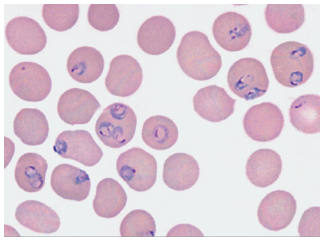

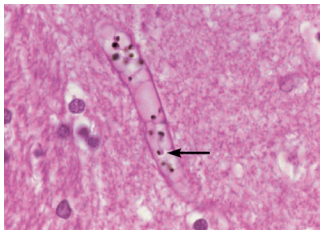

10

Malaria

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Introduction

Definition

Malaria is an infectious disease caused by coccidian protozoa of the genus *Plasmodium*, and transmitted by infected female anopheline mosquitoes. *Plasmodium* sp infecting humans include *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, and *Plasmodium ovale*. The 4 species differ in geographic distribution, microscopic appearance, and clinical features.

Synonyms

Infections with *P. vivax*, *P. falciparum*, *P. malariae*, and *P. ovale* are known respectively as vivax or benign tertian malaria, falciparum malaria, quartan malaria, and ovale malaria. The terms tertian and quartan describe the usual periodicity of the fever.

General Considerations

In the mid-19th century Meckel and others discovered a black granular substance in the blood and tissues of patients with malaria.[1] In 1847, Meckel observed that the granules lay within protoplasmic masses which Afanasiev, in 1879, suggested were the cause of malaria. In 1880, Laveran, a French army surgeon in Algeria, observed malarial pigment in plasma and leukocytes and clear bodies within erythrocytes. As the irregularly shaped hyaline bodies grew, Laveran noted that the erythrocytes paled and pigment formed within them. Later he observed male gametes form by exflagellation and described the male and female gametes, the trophozoite, and the schizont forms. In 1886, Machiafava and Celli gave the generic name *Plasmodium* to the parasite. Most early observations of malarial parasites were made on unstained specimens since methylene blue and eosin stains were not applied to blood smears until 1891 by Romanovsky; a forerunner of today's Wright stain.

In 1895, Ross, a British medical officer in India, hypothesized that the mosquito is the intermediary host of the malarial parasite and began dissecting various species of mosquitoes. In 1897 he found malarial parasites in the stomach wall of *Anopheles* mosquitoes, and in 1898 described the complete life cycle of the parasite that causes avian malaria, and the *Culex* mosquito vector. For his research Ross received the Nobel prize in 1902. Grassi et al later proved that anopheline mosquitoes transmit malaria to humans.[2]

In 2002, researchers sequenced the genomes of *P. falciparum* [3] and one of its vectors, *Anopheles gambiae*.[4]

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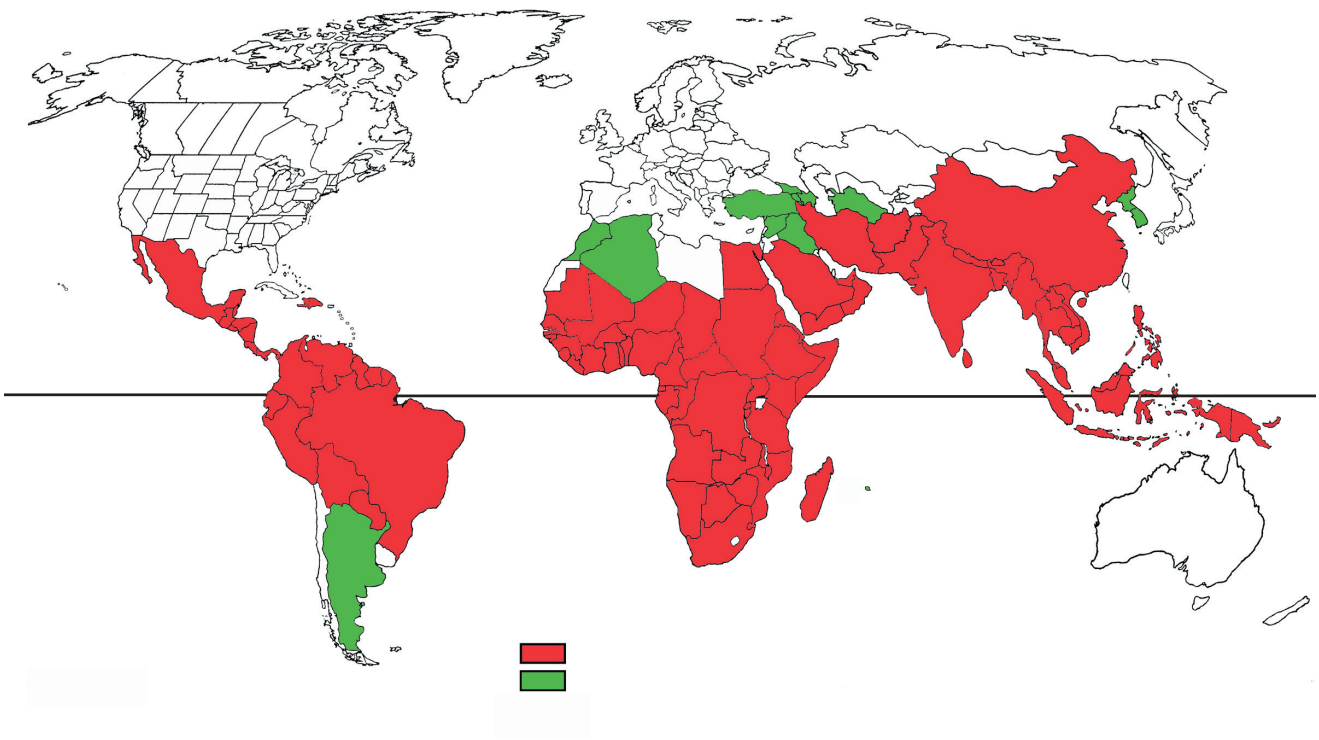

Fig 10.1
Countries endemic for malaria. Regions shown in green are endemic for *Plasmodium vivax* only.

Epidemiology

Malaria is endemic in tropical and subtropical regions of Africa, Asia, Central and South America, and Oceania, and on the West Indies island of Hispaniola (Fig 10.1).[5,6] Rates of infection vary depending on insect vectors, climate, environmental conditions, the local species of *Plasmodium* and its susceptibility to antimalarial drugs, and the genetic composition, acquired immunity, and behavior of human hosts. Malaria is more prevalent in rural areas, but incidence among urban populations is increasing.

Malaria is one of the most prevalent infectious diseases, with over 40% of the world's population at risk. Approximately 300 million cases of clinical malaria are reported worldwide each year, with more than a million fatalities. Almost 90% of fatalities are in sub-Saharan Africa, where young children are most affected. According to WHO estimates, there are approximately 270 million cases of malaria in Africa per year, and an estimated 5% of African children die of malaria-related illness before their fifth birthday.[6] In areas of the world with high infection rates, children, pregnant women in the second and third trimesters and during the early postpartum period,[7] as well as nonimmune visitors are at greatest risk of fatal malarial infection. In areas where infection rates are lower, the risk is equal for everyone. Although residents of endemic areas acquire some immunity, repeated infections cause tremendous loss of life and productivity. *Plasmodium falciparum* is the second most common species infecting humans, it is found almost exclusively in tropical areas and is the cause of the most severe forms of malaria, and most deaths. *Plasmodium malariae* is rare now but was common in Europe at one time, and *P. ovale*, the rarest species, is found in Africa and Southeast Asia. *Plasmodium vivax* is the most common of all species of plasmodia and has a wide geographical distribution, including some temperate regions, but causes milder disease.

In nonendemic areas, most cases of malaria develop in immigrants and travelers from endemic regions. However, anywhere anopheline mosquitoes are present, the potential exists for reintroduction of malaria, even in temperate climates where the disease has been eradicated. There are rare reports of stowaway mosquitoes transmitting malaria to people who live near airports.[8] Malaria was once common in North America, especially in the Mississippi Valley, but on average, one case per year is now reported in the United States. Most of these patients were infected during travel to

Table 10.1
Morphologic features of *Plasmodium* sp in thin peripheral blood films.

	P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Erythrocyte	Usually enlarged, pale, irregularly shaped	Normal size	Normal size or slightly smaller	Usually enlarged, frequently oval or fimbriated
Stippling (pink-staining granules)	Schüffner's dots	Coarse Maurer's dots rarely observed	Ziemann's dots rarely observed	Schüffner's dots
Incidence of multiply infected erythrocytes	Occasional	Frequent	Rare	Occasional
Trophozoites	Tenuous and ameboid forms, large, occasional double chromatin dots	Small ring forms frequent, 2 chromatin dots, marginal forms common	Frequent band forms, rare double chromatin dots	Compact, large, sometimes slightly ameboid, occasional double chromatin dots
Schizonts	Less numerous than trophozoites, pigment clumped in mature forms	Rare, clumped pigment in immature and mature forms	Often in great numbers, pigment clumped in mature forms	May be frequent, pigment clumped in mature forms
Number of merozoites in mature schizont	16 (12-24)	8-24 (more if multiply infected erythrocyte)	8 (6-12)	8 (4-16)
Merozoites	Small, clustered	Very small	Large, frequent rosette pattern around central pigment	Very large, frequent rosette pattern around central pigment
Pigment	Yellow-brown	Dark brown	Dark brown	Brown
Gametocytes	Usually round, fill most of enlarged erythrocyte	Rare, crescent- or sausage-shaped	Usually round, fill most of normal-sized or slightly smaller erythrocytes	Round or oval, fill most of enlarged erythrocyte

endemic areas (imported malaria). Occasionally, local mosquitoes become infected by gametocytemic individuals and pass the infection to nontravelers (autochthonous or introduced malaria).

Infectious Agent

Morphologic Description

In peripheral blood, all species of *Plasmodium* that infect humans are composed of chromatin and cytoplasm, may or may not contain pigment, and are present in 3 forms: trophozoites (growing forms), schizonts (dividing forms), and gametocytes (sexual forms).

Trophozoites range from small young ring forms to mature forms with chromatin that is still undivided. In all four types of malaria, young ring forms, usually indistinguishable among species, may be observed in peripheral blood.

In immature schizonts, division has just begun. In mature schizonts, division of chromatin and cytoplasm is complete, pigment is clumped in a single mass, and cytoplasm is separated into distinct masses. The number of merozoites (asexual components of schizonts) in a mature schizont varies considerably among the different species of *Plasmodium*.

Gametocytes of *P. falciparum* are elongated or sausage-shaped; gametocytes of all other species are round, with a single chromatin mass and compact cytoplasm.

Morphologic features of *Plasmodium*, as seen on thin blood films that allow determination of species, are summarized in Table 10.1.[9,10]

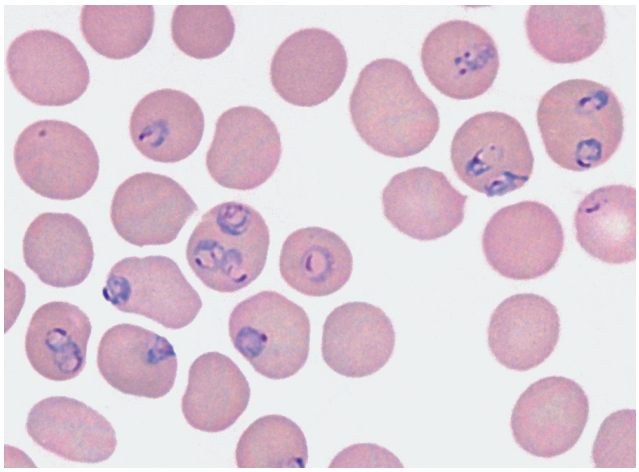

Figure 10.2
Heavy parasitemia (30%) in patient who died of falciparum malaria. Infected erythrocytes are normal size. Giemsa. Original magnification x330

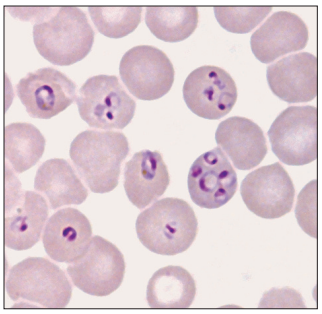

Figure 10.3
Five ring trophozoites in single erythrocyte. Multiply infected erythrocytes are very common in *Plasmodium falciparum* infections. Giemsa. Original magnification x330

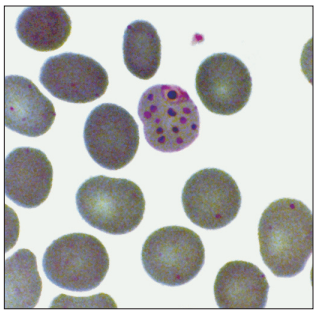

Figure 10.4
Pink-staining Maurer's dots in erythrocyte cytoplasm stained at pH 7.4. Giemsa. Original magnification x300

Plasmodium falciparum (Figures 10.2 to 10.10)

Erythrocytes infected with *P. falciparum* are of normal size. Parasitemia is often much greater in falciparum malaria than in the other 3 types. Multiply infected erythrocytes are common, especially in heavy infections (Figs 10.2 to 10.4). Infection by asexual parasites from the older ring trophozoite stage onward causes erythrocytes to be stippled with pink Maurer's dots of various shapes and sizes. Maurer's dots are rarely observed unless they are overstained or stain is alkaline (Fig 10.4).

Young ring trophozoites (Fig 10.5a) of *P. falciparum* are smaller than those of other species (approximately one fifth the diameter of an erythrocyte) and more likely to have double chromatin dots (Fig 10.5b). Three and even 4 chromatin dots may rarely be observed. Young ring trophozoites have delicate, thread-like cytoplasm; very young trophozoites usually contain no pigment. They may be round, rectangular, flame-shaped, or narrow band-shaped (Fig 10.5c). Flattened marginal forms and bridge forms are more common than in other species (Fig 10.5d). Older ring trophozoites are slightly larger and have more cytoplasm and chromatin (Fig 10.5e). Traces of tiny pigment granules may give the cytoplasm a yellowish tinge, and there may be basophilic stippling (blue dots) (Figs 10.5f). It is characteristic in falciparum malaria to observe only ring forms of the asexual cycle, with no older trophozoites or schizonts, in peripheral blood. Mature trophozoites have small, compact, light-staining cytoplasm, a larger chromatin dot, and a small, dense, nearly black clump of pigment (Fig 10.5g). Clumping of pigment at this stage is an exclusive characteristic of *P. falciparum*.

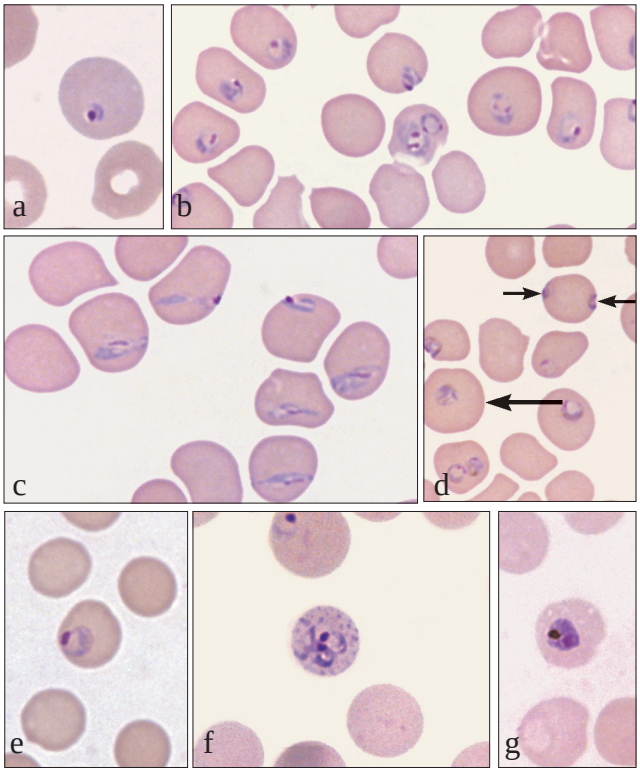

Figure 10.5 a,b,c,d,e,f,g
Plasmodium falciparum trophpzoites. Giemsa. Original magnifications all x333 except e x290.
a. Small ring trophozoite, no pigment. b. Double chromatin dots. c. Narrow band forms. d. Flattened marginal forms (arrows). e. Older ring trophozoite. f. Erythrocyte basophillic stippling. g. Mature trophozoite with pigment lump in cytoplasm.

Schizonts of *P. falciparum* are rarely observed in peripheral blood, even in heavy infections. During schizogony, the nucleus divides and the cytoplasm breaks apart. In immature schizonts, the chromatin dots and dark brown pigment clumps are dividing and are far more noticeable than the small amount of clear cytoplasm surrounding them (Figs 10.6a & 10.6b). Mature schizonts contain 8 to 24 minute merozoites that are often arranged in a rosette pattern (Fig 10.6c). When more than one schizont infects an erythrocyte, there may be more merozoites. Each schizont in a multiply infected erythrocyte has a separate clump of dark brown pigment. When the schizonts reach maturity, infected erythrocytes burst, releasing merozoites that enter new erythrocytes to begin another generation.

Immature *P. falciparum* gametocytes are seldom observed in peripheral blood (Figs 10.7a & 10.7b). The earliest forms are small, compact, and round. Pigment is scattered throughout the cytoplasm and the nucleus is sometimes stretched along one side. As it matures, the gametocyte becomes elongate, angular, or oval, and the chromatin tends to migrate toward the center. Fully matured gametocytes, which are more commonly seen than younger forms, are usually crescent- or sausage-shaped. Macrogametocytes are usually slightly longer, more slender with pointed ends, and more deeply stained than microgametocytes (Fig 10.7c). Macrogametocytes have dense blue cytoplasm and a small, compact, red or magenta chromatin mass lying in or near the center or near one of the poles. Pigment closely adheres to the chromatin in separate grains, surrounding it or covering it completely. Microgametocytes are sausage-shaped with rounded ends and have pale, often grayish-blue or pink cytoplasm and loose, irregularly scattered chromatin granules (Fig 10.7d). Abundant small, brownish rodlets and granules of pigment occupy the central portion of the parasite. The ends of mature macro- and microgametocytes are usually clear and pigment-free. Infected erythrocytes stretch as gametocytes grow longer. Frequently, the residuum of erythrocytic material filling the concave side of the parasite develops a faint, bowshaped projecting rim. Sometimes the remains of the erythrocyte appear as a red zone around the gametocyte.

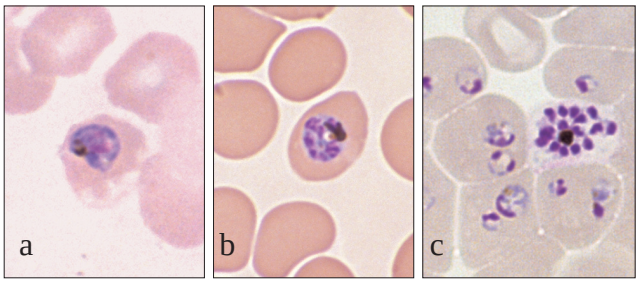

Figure 10.6 a,b,c
Plasmodium falciparum schizont forms in thin peripheral blood smears, Giemsa. Original magnifications, a & b Immature schizonts. x330 c. Mature schizont with 15 merozoites and central pigment clump. x300

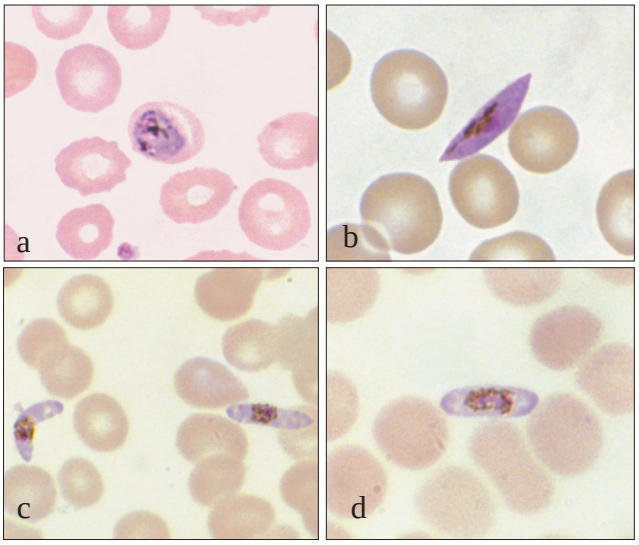

Figure 10.7 a,b,c,d
Immature and mature *Plasmodium falciparum* gametocyte forms in thin peripheral smears, Giemsa. Original magnifications. Immature gametocytes (a & b). a. Large round form. x400 b. Elongated shape with pointed ends containing chromatin mass and pigment. x300 c. Sausage-shaped mature macrogametocyte with pointed ends, contains compact chromatin and pigment. x450 d. Sausage-shaped mature microgametocyte has rounded ends and contains diffuse pigment and chromatin. x450

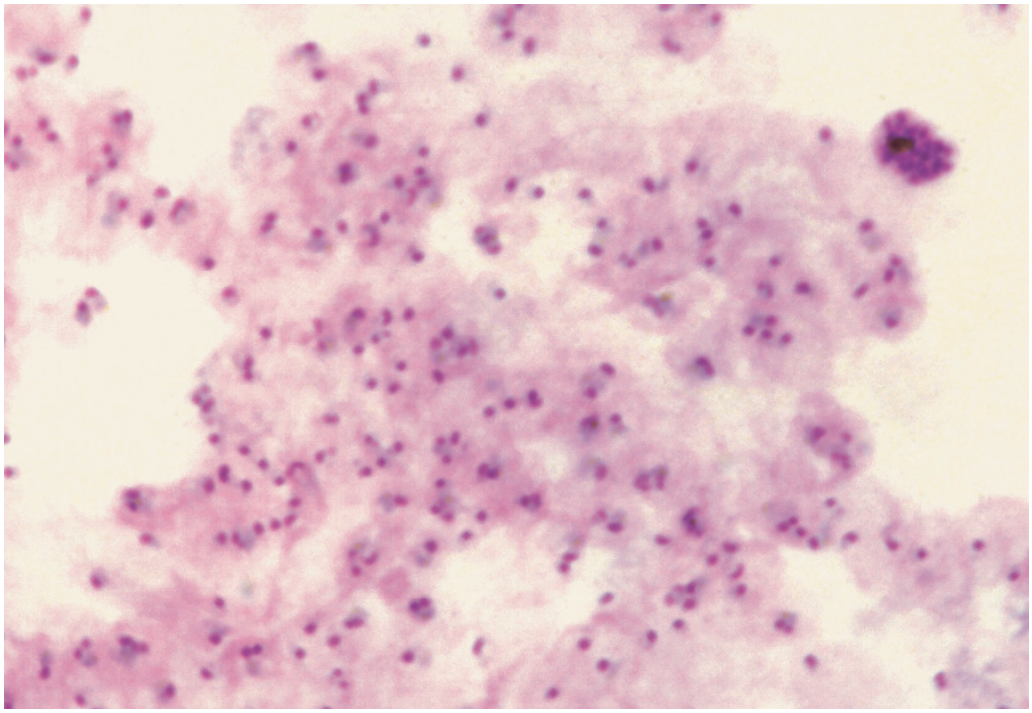

Figure 10.8
Plasmodium falciparum in thick blood film from patient with 43% parasitemia. Trophozoites have 1 or 2 large chromatin masses and small, compact cytoplasm. Some have faint pigment. Giemsa. Original magnification x290

Thick blood films demonstrate masses of trophozoites (Fig 10.8), a mature schizont with central clumped pigment surrounded by merozoites in rosette pattern (Fig 10.9), and sausage-shaped gametocytes (Fig 10.10).

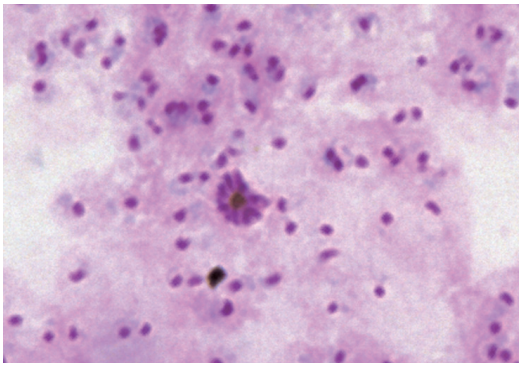

Figure 10.9
Mature *Plasmodium falciparum* schizont in thick blood film with clumped pigment in center and merozoites in rosette pattern. Giemsa. Original magnification x330

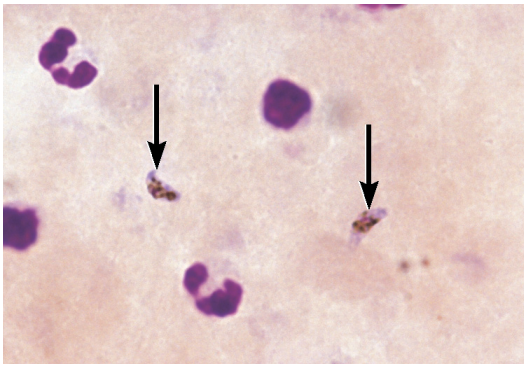

Figure 10.10
Two sausage-shaped *Plasmodium falciparum* gametocytes in thick blood film. Giemsa. Original magnification x300

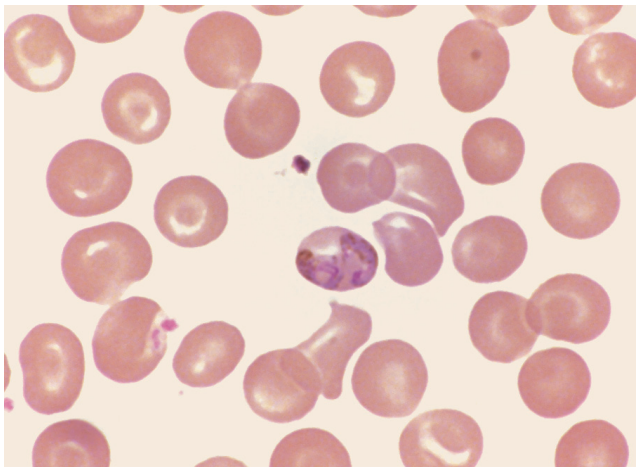

Figure 10.11
Erythrocyte with 2 trophozoites, a rare finding in *Plasmodium malariae* infections. Giemsa. Original magnification x290

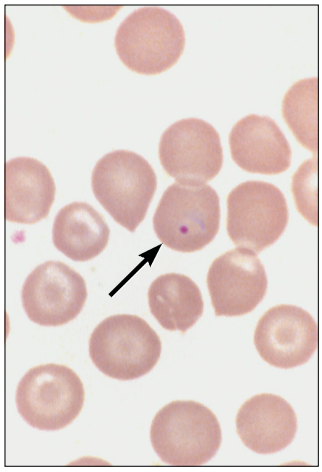

Figure 10.12
Early ring trophozoite (arrow) of *Plasmodium malariae* with red chromatin dot and faintly stained blue cytoplasm in normal-sized erythrocyte. Giemsa. Original magnification x290

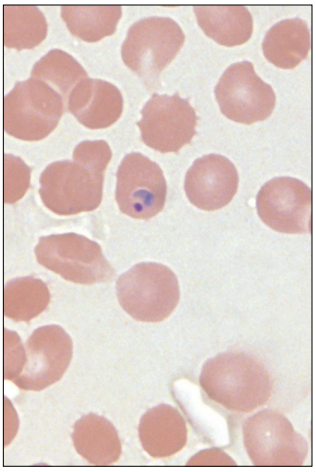

Figure 10.13
Ring trophozoite of *Plasmodium malariae* with red chromatin dot and prominent blue cytoplasm in normal-sized erythrocyte. Giemsa. Original magnification x300

Plasmodium malariae (Figures 10.11 to 10.17)

Erythrocytes infected with *Plasmodium malariae* are normal-sized or slightly smaller. Parasitemia in *P. malariae* infections is generally less than 2%. When stained at pH 7.5, stippling with various sizes of pale pink, spherical Ziemann's dots may be seen. However, this stippling is less distinct than in *P. falciparum* and *P. vivax* infections and too rarely observed to be diagnostically significant. Multiply infected erythrocytes are rare (Fig 10.11).

Very young ring trophozoites (Fig 10.12) usually contain no pigment. Young ring trophozoites (Fig 10.13) are roughly the size of *P. vivax* trophozoites, but some are smaller and some have a broader circle of cytoplasm. Double chromatin dots are rare. Some trophozoites contain a vacuole that gives the parasite the appearance of a basket with a handle (Fig 10.14a). Growing trophozoites are usually compact and angular, round, ovoid, or band-shaped. Band forms are more frequently observed in *P. malariae* than in other species (Figs 10.14b & 10.14c). The chromatin in growing or older trophozoites may be round or streaky and frequently looks stretched. The pigment is darker than that of *P. vivax* and is often round and arranged peripherally, sometimes opposite the elongated nucleus. The large pigment granules may have a yellowish edge that gives the blue cytoplasm a greenish hue, a characteristic of *P. malariae* that distinguishes it from *P. vivax*. Trophozoites of *P. malariae* rarely become ameboid like *P. vivax*, but retain their original shape until they mature (Fig 10.14d).

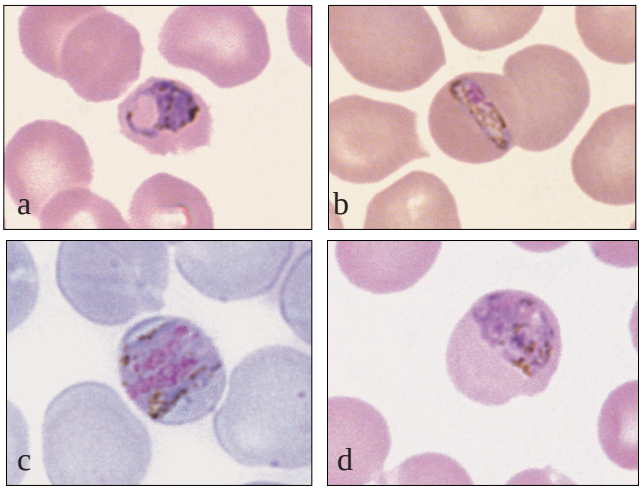

Figure 10.14 a,b,c,d
Plasmodium malariae trophozoites, thin peripheral blood smear, Giemsa. Original magnifications a. Basket appearance. x330 b. Early band form. x450 c. Mature band form. Blue staining of erythrocyte cytoplasm as seen is unusual. x450 d. Mature rounded compact form. x300

Erythrocytes containing schizonts of *P. malariae* usually do not withdraw from peripheral blood, as they do in vivax malaria. Therefore, erythrocytes with schizonts of P. *malariae* are often observed in great numbers in peripheral blood. Immature schizonts can be so dark and dense that it is difficult to differentiate the divisions of chromatin within the heavily pigmented cytoplasm (Fig 10.15a). The divisions are frequently uneven in size and shape. The pigment remains scattered throughout the cytoplasm until shortly before complete division of the chromatin. Mature schizonts (Figs 10.15b & 10.15c) consist of 6 to 12 (average 8) large merozoites arranged peripherally in a rosette pattern around a central clump of dark brown pigment.

Gametocytes (Fig 10.16a & 10.16b) of *P. malariae* are seldom observed in peripheral blood. It may be difficult to differentiate young gametocytes from round, compact trophozoites. Mature gametocytes of *P. malariae* are spherical or oval, larger than mature trophozoites, but smaller than gametocytes of *P. vivax*. They usually fill or nearly fill a normal-sized erythrocyte. Macrogametocytes (Fig 10.16a) and microgametocytes (Fig 10.16b) are roughly the same size and have the same staining qualities. The chromatin is eccentric in macrogametocytes and central in microgametocytes. The abundant, prominent, dark brown, coarse pigment grains are darker than in *P. vivax*, particularly in macrogametocytes.

Thick blood films demonstrate two trophozoites in Fig 10.17a, an immature schizont in Fig 10.17b, and a mature schizont in Fig 10.17c.

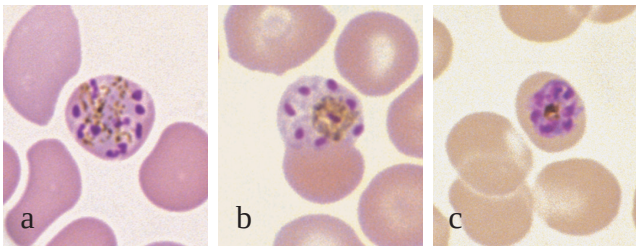

Figure 10.15 a,b,c
Plasmodium malariae schizont forms in thin peripheral blood smear. Giemsa. Original magnifications. a. Immature schizont. Chromatin and cytoplasm still dividing, light brown granular pigment diffuse. x300 b. Nearly mature schizont with eight merozoites forming rosette. Chromatinc division is complete and pigment is clumped. x330 c. Mature schizont with seven merozoites rosette with clumped central pigment. x330

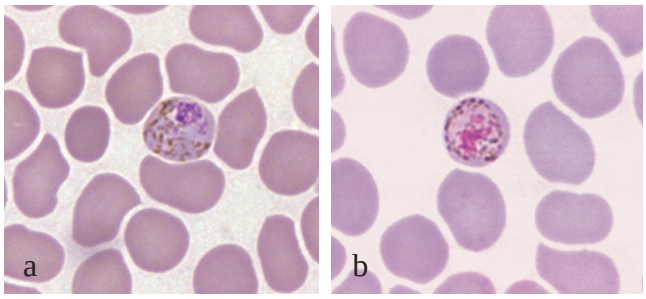

Figure 10.16 a,b
Plasmodium malariae gametocyte forms in thin peripheral blood smears. Giemsa. Original magnifications. a. Macrogametocyte with eccentric chromatin. x330 b. Microgametocyte with central chromatin. x400

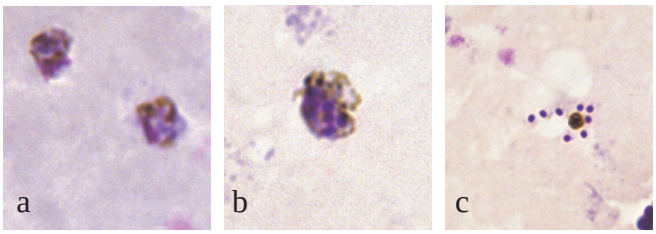

Figure 10.17 a,b,c
Plasmodium malariae forms in thick peripheral blood smears. Giemsa. Original magnifications x330. a. Two trophozoites with blue cytoplasm, red chromatin and brown pigment. b. Immature schizont with several chromatin masses, undivided cytoplasm and brown pigment. c. Mature schizont with 8 merozoites forming rosette and clumped brown pigment.

Plasmodium ovale (Figures 10.18 to 10.24)

Erythrocytes infected with *P. ovale* are initially normal-sized (Fig 10.18a–10.18c) but eventually enlarge nearly as much as in *P. vivax* infection (Figs 10.19a & 10.19b). Parasitemia in *P. ovale* infections is generally less than 2%. Infected erythrocytes are often oval and fimbriated and may be stippled with Schüffner's dots (Fig 10.19b). Although fimbriated (Fig 10.20a & 10.20b) and elongated erythrocytes (the ovalocytes, for which this species is named) (Fig 10.20) are artifacts that occur in dry, fixed, thin films, they are of great diagnostic value. Multiply infected erythrocytes are occasionally observed (Fig 10.21a).

Very young ring trophozoites of *P. ovale* usually contain no pigment (Fig 10.19a). Young trophozoites are about the same size as *P. vivax* and have a large chromatin dot and a few may have double chromatin dots (Fig 10.18c). Some have a heavy cytoplasmic circle, like *P. malariae*. In growing *P. ovale* trophozoites, the chromatin is solid and compact; the cytoplasm is only slightly ameboid (Fig 10.21b), has few vacuoles and less commonly form bands (Fig 10.21c & 10.21d). Mature trophozoites are round and centrally located. Infected erythrocytes often become oval,

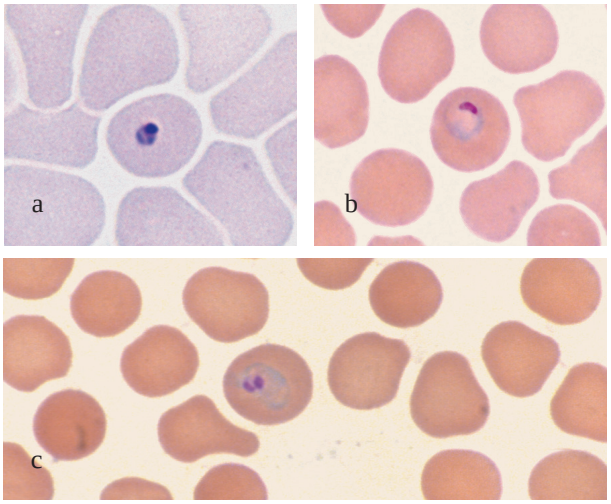

Figure 10.18 a,b,c
Ring forms of *Plasmodium ovale* in normal sized erythrocytes. Giemsa. Original magnifications. a. Small early ring trophozoite, chromatin dot, no pigment. x330 b. Older ring trophozoite, red chromatin dot. x290 c. Ring trophozoite with 2 chromatin dots. x290

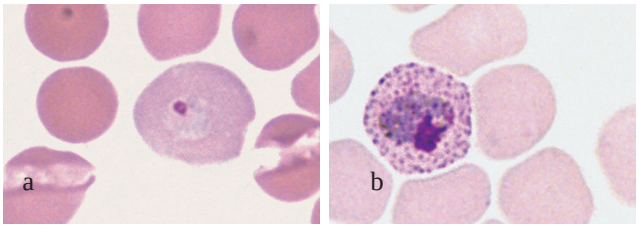

Figure 10.19 a,b
Trophozoites of *Plasmodium ovale* in enlarged erythrocytes. Giemsa. Original magnifications x330. a. Ring trophozoite lacks pigment. b. Mature trophozoite contains Schüffner's dots.

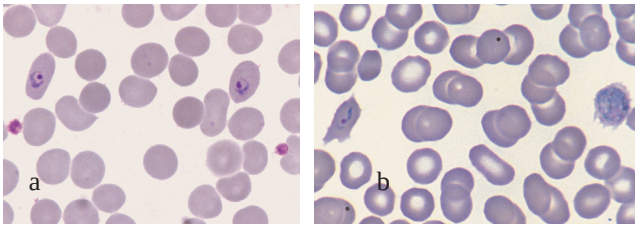

Figure 10.20 a,b
Oval and fimbriated erythrocytes infected with *Plasmodium ovale*. Giemsa. Original magnifications. a. Two ring trophozoites in oval erythrocytes. x330 b. Ring trophozoites in fimbriated oval erythrocyte (left) and fimbriated round erythrocyte (right) with macrogametocyte. x290

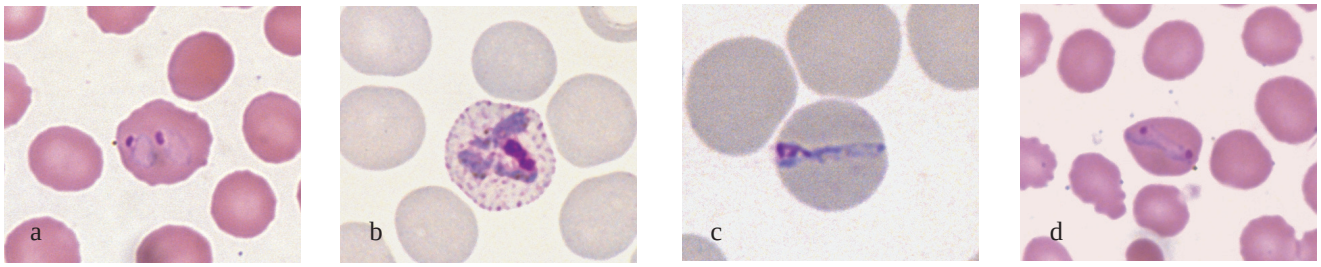

Figure 10.21 a,b,c,d
Various forms of *Plasmodium ovale* trophozoites. Giemsa. Original magnifications. a. Multiple infected erythrocytes. x300 b. Slight ameboid configuration with Schüffner's dots. x290 c. Elongate thin band shaped trophozoite. x330 d. Elongate band shaped trophozoite with 2 chromatin dots. x300

spindle-, or pear-shaped, with ragged points or fimbriations on one or both ends. Schüffner's dots may extend to the tips of the fimbriations. In this and later stages, *P. ovale* closely resembles *P. malariae*, except that its pigment is lighter and less conspicuous.

Immature schizonts (Figs 10.22a to 10.22c) of *P. ovale* are usually larger than those of *P. malariae*. Erythrocytes that contain them are frequently fimbriated and oval or elongated. Mature schizonts (Fig 10.22d) consist of 4 to 16 (average 8) very large merozoites, usually in a rosette pattern.

Mature macrogametocytes with eccentric chromatin mass (Fig 10.23a) and mature microgametocyte with central chromatin mass (Fig 10.23b) of *P. ovale* are round and slightly smaller than those of *P. vivax* and contain Schüffner's dots. Pigment rods are brown-black with a greenish tinge, distinctively arranged in a concentric pattern at right angles to the radius. They are more numerous at the periphery. Mature gametocytes fill or nearly fill an enlarged erythrocyte.

Thick blood films demonstrate an ameboid trophozoite in Fig 10.24a, a mature schizont with 8 merozoites in Fig 10.24b, and a gametocyte in Fig 10.24c.

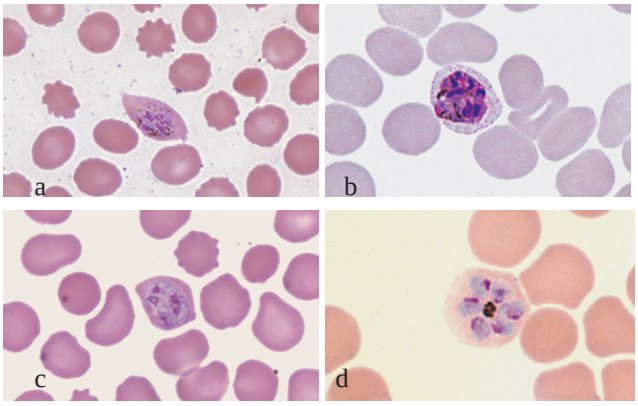

Figure 10.22 a,b,c,d
Plasmodium ovale schizonts. Original magnifications. a. Immature schizont, chromatin and cytoplasm dividing. x300 b. Pigment beginning to clump, 7 chromatin masses. x330 c. Late immature schizont fills most of erythrocyte. x300 d. Mature schizont in rosette pattern with 6 merozoites. x290

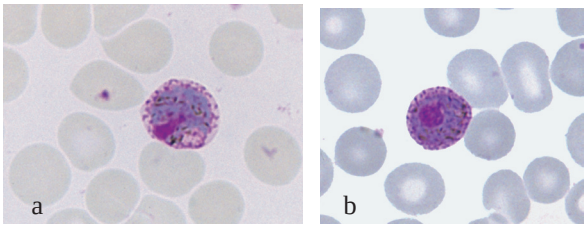

Figure 10.23 a,b
Mature *Plasmodium ovale* gametocytes.in thin peripheral blood stains. Giemsa. Original magnification x330. a. Mature macrogametocyte with Schüffner's dots and eccentric chromatin mass. b. Mature microgametocyte with Schüffner's dots and large central mass of chromatin.

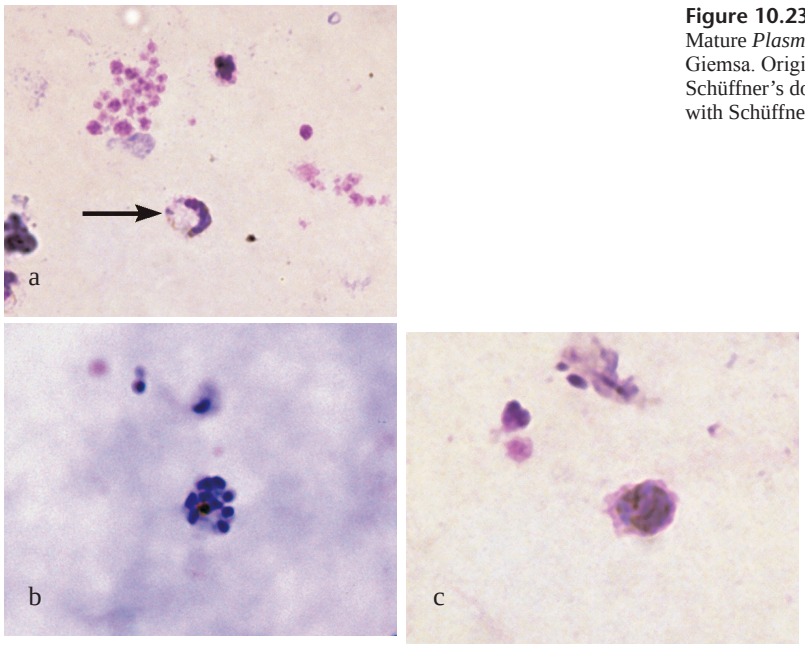

Figure 10.24 a,b,c
Thick blood film of *Plasmodium ovale*. Original magnifications. a. Ameboid trophozoite. x300 b. Mature schizont. x330 c. Gametocyte. x300

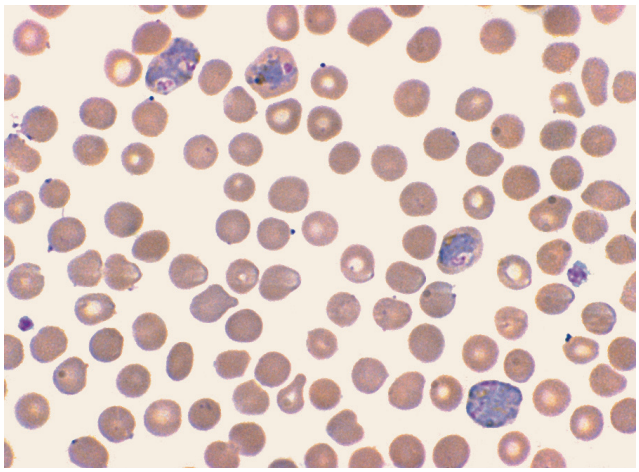

Figure 10.25
Heavy parasitemia (approximately 4%) in patient infected with *Plasmodium vivax*. Giemsa. Original magnification x250

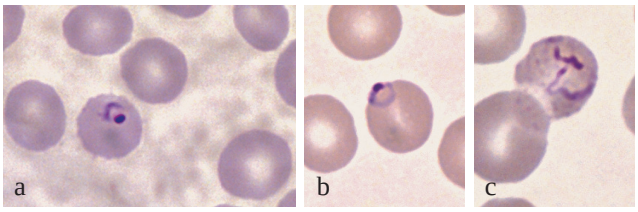

Figure 10.26 a,b,c
Young trophozoites of *Plasmodium vivax* in normal sized erythrocytes. Original magnifications. a. Young ring trophozoite, lack pigment. x300 b. Marginal ring trophozoite. x 300 c. Tenuous trophozoite. x330

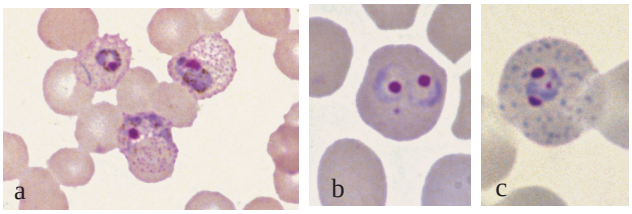

Figure 10.27 a,b,c
Multiply infected enlarged erythrocytes with *Plasmodium vivax*. Giemsa pH 7.2. Original magnifications. a. Schüffner's dots in 3 older trophozoites. x290 b. Two ring trophozoites. x450 c. Trophozoite with 2 chromatin dots, cytoplasm of erythrocyte has basophilic stippling. x450

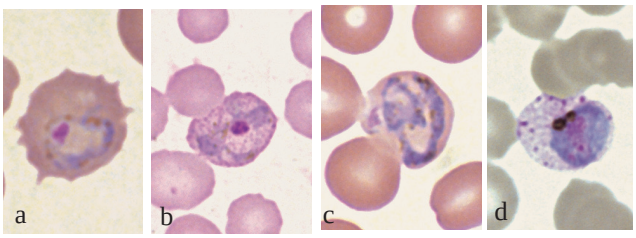

Figure 10.28 a,b,c,d
Various forms of *Plasmodium vivax* trophozoites. Original magnifications. a.Older trophozoite in fimbriated erythrocyte. x450 b. Ameboid trophozoite with Schüffner's dots. x300 c. Ameboid trophozoite. x330 d. Mature trophozoite. x300

Plasmodium vivax (Figures 10.25 to 10.31)

Erythrocytes infected with *P. vivax* eventually enlarge, become pale, and irregularly shaped. Parasitemia in *P. vivax* infections is generally less than 2%, but may be higher (4% in Fig 10.25 & 10.26). Parasitized oval erythrocytes are common. The cytoplasm of parasitized erythrocytes may be stippled with small, fairly uniform pink granules called Schüffner's dots which are evenly distributed throughout the part of the erythrocyte that is not occupied by the parasite (Fig 10.27a); Schüffner's dots often become more pronounced and more deeply stained as the parasite grows, and are best demonstrated by careful staining at pH 7.2 preventing prolonged washing which obliterates them. Schüffner's dots are more numerous and less coarse than the Maurer's dots sometimes seen in falciparum malaria. Multiply infected erythrocytes (Fig 10.27b) and double chromatin dots (Fig 10.27c) are occasionally observed, and often different stages of *P. vivax* appear simultaneously because of asynchronous maturation within a brood, and alternate day maturation of separate broods.

Young ring trophozoites of *P. vivax* are large (approximately one third the diameter of an erythrocyte,) consist of blue cytoplasm and a heavy red chromatin dot in the center or at the periphery. Very young ring trophozoites are usually unpigmented (Fig 10.26a). Marginal ring trophozoites may be observed in some erythrocytes (Fig 10.26b). As trophozoites grow their cytoplasm thickens, the chromatin mass enlarges, pigment granules develop, and the host's cytoplasms occasionally fimbriates (Fig 10.28a). Growing trophozoites develop one or more vacuoles; tenuous pseudopodial processes sometimes give them an ameboid appearance (Figs 10.28b &10.28c). In young forms hemozoin (hematin) , pigment granules may be indistinguishable as separate granules or rods due to the yellowish tinge they lend to the cytoplasm. In mature forms pigment granules are small, yellowish-brown, and angular or rod-shaped. At the end of vegetative growth, pseudopodia are drawn in and trophozoites become compact, with an irregular outline and mottled cytoplasm (Fig 10.28d). The single chromatin mass is compact and usually becomes located near the periphery. Full-grown *P. vivax* trophozoites are larger than those of other species and practically fill an enlarged erythrocyte.

Schizonts of *P. vivax* tend to leave peripheral circulation and thus are less frequently found than trophozoites in peripheral blood. Immature schizonts are produced by division of chromatin (Figs 10.29a to 10.29c). When division is complete, the chromatin masses become smaller and more rounded and are surrounded by a mass or circle of cytoplasm. Mature schizonts consist of 12 to 24 small, clustered merozoites (average 16, but as few as 9 have been described) (Figs 10.29d & 10.29e). The yellow-brown pigment clumps (sometimes arranged loosely into a single mass) (Fig 10.29d) are often localized in the center of the cluster of merozoites forming "rosettes" (Fig 10.29e).

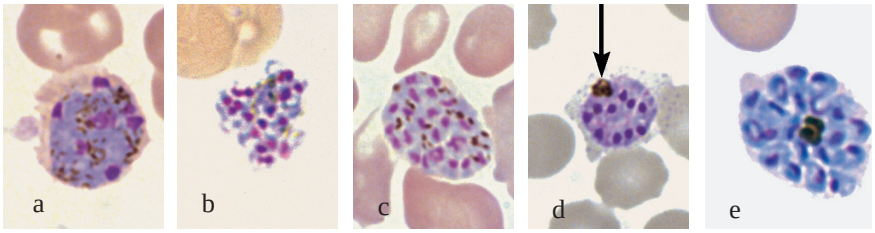

Figure 10.29 a,b,c,d,e
Plasmodium vivax schizonts. Giemsa. Original magnifications. a. Early immature, 7 chromatin masses. x450 b. Extracellular, immature. x330 c. Late immature with 24 merozoites. x450 d. Mature schizont with 10 merozoites and yellow-brown pigment (arrow). x330 e. Mature schizont with 20 merozoites. x330

Gametocytes of *P. vivax* develop from released merozoites in the circulation of the deep organs before release to the general circulation, for that reason young forms are only infrequently found in the peripheral blood. Immature gametocytes are rounded and have homogeneous cytoplasm, often with a vesicular area around the chromatin mass (Fig 10.30a). They are difficult to distinguish from growing and mature trophozoites whose pseudopodia have drawn in prior to desiccation or with maturation. Mature gametocytes expand the erythrocytes (Figs 10.30b to 10.30e), exhibit pigment granules that become evenly distributed throughout the cytoplasm and are more numerous than in trophozoites. Slightly immature macrogametocytes (female) of *P. vivax* can be distinguished from mature trophozoites by their larger size, circular to ovoid contour, homogenous cytoplasm with larger and darker brown pigment granules (often more numerous than in trophozoites) and lack of vacuoles. Mature macrogametocytes (Figs 10.30b & 10.30c) have densely blue-staining, homogeneous cytoplasm, a compact chromatin mass that is deep red or magenta near the periphery and sometimes surrounded by a colorless vesicular area. Mature microgametocytes (male) (Figs 10.30d & 10.30e) are usually also found in enlarged erythrocytes. Their reticular chromatin mass stains lightly, and is round or stellate and larger than in any other stage, sometimes extending across the body of the parasite in a broad spindle. The reticular chromatin mass of mature microgametocytes of *P. vivax* is centrally located and it is often surrounded by a large unstained vesicular area. Pigment grains and rods are usually lighter than in macrogametocytes.

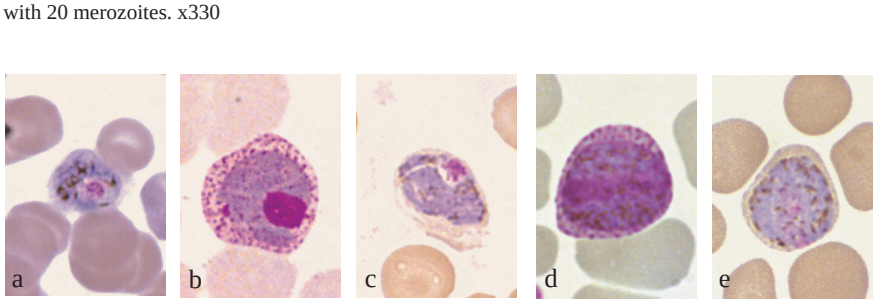

Figure 10.30 a,b,c,d,e
Plasmodium vivax gametocytes in thin peripheral blood films. Giemsa. Original magnifications. a. Immature gametocyte. x300 b. Mature macrogametocyte. x330 c. Mature oval macrogametocyte. x330 d. Mature microgametocyte. x450 e. Mature microgametocyte. x300

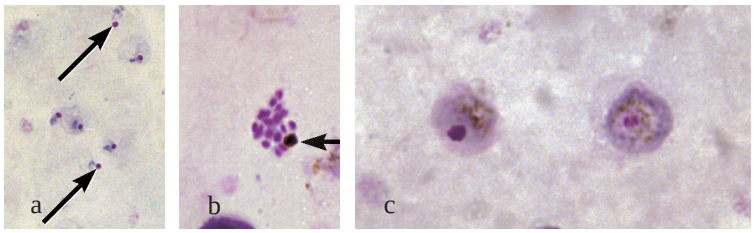

Figure 10.31 a,b,c
Thick films of *Plasmodium vivax.* Giemsa. Original magnifications. a. Ring trophozoites (arrows). x290 b. Mature schizont with yellow-brown pigment (arrow). x330 c. Mature gametocyte (left) immature gametocyte (right). x300

Thick blood films demonstrate several ring trophozoites (Fig 10.31a), a mature schizont with 16 merozoites (Fig 10.31b) and a mature gametozoite (left) and an immature gametocyte with vacuole surrounding chromatin mass (right) (Fig 10.31c).

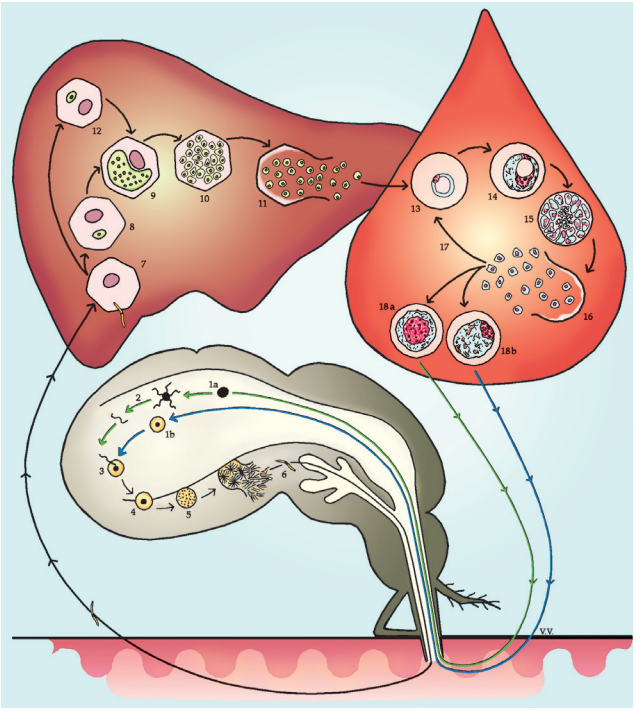

Figure 10.32
Life cycle of malarial parasites. Life cycle of *Plasmodium* sp. 1. Mosquito ingests sexual-stage malarial parasites (micro- and macrogametocytes). 2. Microgametocyte exflagellates in mosquito's gut. 3. Exflagellated microgamete fertilizes macrogamete, forming a zygote. 4. Zygote develops into ookinete that penetrates epithelial cells of mosquito's midgut. 5. Ookinete grows into oocyst. 6. Thin motile sporozoites develop, disperse through mosquito's body cavity, lodge in salivary glands, and are inoculated into host during subsequent blood meal. 7. In human host, sporozoites leave blood and infect hepatocytes. 8-10. Sporozoites undergo schizogony, forming tissue schizonts containing many exoerythrocytic merozoites. 11. Hepatocytes rupture and release merozoites into bloodstream. 12. *Plasmodium vivax* and *P. ovale* hypnozoites remain dormant in liver; subsequent schizogony causes relapse. 13. Asexual cycle begins when merozoites from liver invade erythrocytes and develop into young ring form trophozoites. 14. Older mature trophozoites are larger and usually more compact. 15. Mature trophozoite undergoes asexual division (schizogony) to produce mature schizont composed of merozoites. 16. Erythrocyte ruptures, releasing merozoites into bloodstream. 17. Released merozoites invade other erythrocytes and begin new round of asexual reproduction. 18. Some merozoites develop into micro- and macrogametocytes within erythrocytes, which are then ingested by mosquito.

Life Cycle and Transmission

The life cycle of malarial parasites is illustrated in Figure 10.32. The sexual reproductive cycle in the mosquito, called sporogony, takes 8 to 35 days.

A female anopheline mosquito (Fig 10.33) taking a blood meal from an infected host ingests malarial parasites, some of which are in the sexual stage of development (micro- and macrogametocytes). The microgametocyte undergoes a process of exflagellation. The nucleus divides into 4 to 8 nuclei, each of which combines with cytoplasm to form a

Figure 10.33
Female Anopheline mosquito.

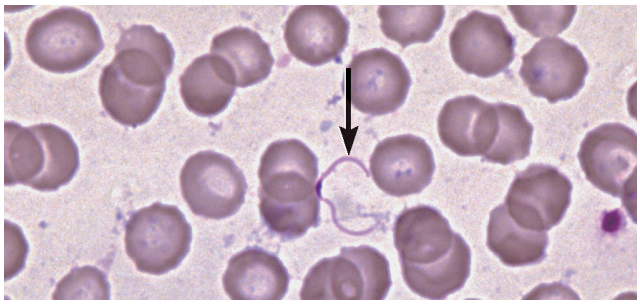

Figure 10.34
Exflagellate form (arrow) of *Plasmodium* in peripheral blood. Giemsa. Original magnification x330

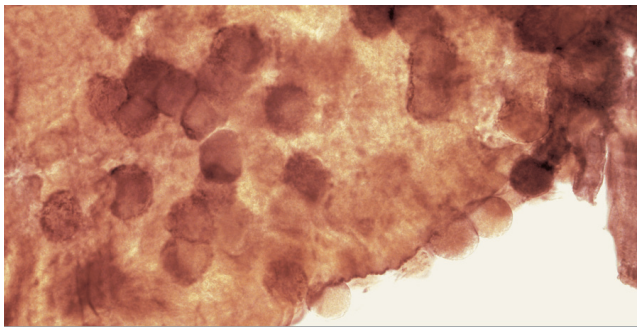

Figure 10.35
Spherical oocysts attached to fragment of infected *Anopheles* mosquito gut. Oocysts are approximately 50 µm in diameter. Unstained. Original magnification x120

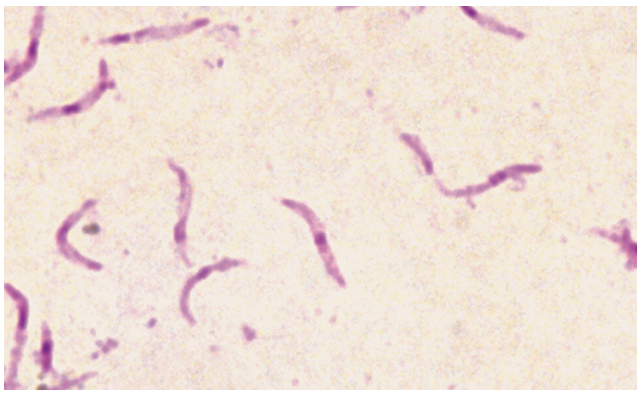

Figure 10.36
Elongated sporozoites, approximately 8 µm to 10 µm by 1 µm, from gut of infected *Anopheles* mosquito. Giemsa. Original magnification x300

long, threadlike flagellum. Exflagellation normally occurs in the mosquito's gut, but the process has been observed in peripheral blood films (Fig 10.34).

Gametocytes develop into micro- and macrogametes in the mosquito's gut. An exflagellated microgamete fertilizes a macrogamete, forming a zygote. The zygote develops into an ookinete that penetrates the epithelial cells of the mosquito's midgut. The ookinete grows into a spherical oocyst in which thin, motile sporozoites develop and disperse throughout the mosquito's body cavity (Figs 10.35 & 10.36). An infected mosquito may contain hundreds of oocysts that produce thousands of sporozoites. Sporozoites that reach the mosquito's salivary glands lodge there and are inoculated into a new host when the mosquito takes another blood meal.

In humans, sporozoites leave the blood and infect hepatocytes. In a process called primary exoerythrocytic schizogony, sporozoites divide and mature over a period of 6 to 16 days, forming primary, or tissue, schizonts containing many exoerythrocytic merozoites. The hepatocytes rupture and release the merozoites into the bloodstream, where they invade erythrocytes. In *vivax* and *ovale* malaria, quiescent forms called hypnozoites remain dormant in the liver and may be released into the blood at any time up to 5 years, causing a recurrence of disease called relapse. P*lasmodium falciparum* and *P. malariae* do not develop hypnozoites and therefore do not relapse. In these 2 species, renewed parasitemia, called recrudescence, is caused by erythrocytic parasites that have remained in circulation at subclinical or asymptomatic levels. *Plasmodium falciparum* can recrudesce for 1 to 2 years and *P. malariae* for 30 or more years.

The asexual erythrocytic cycle begins when merozoites released from the liver invade erythrocytes in the bloodstream and develop into trophozoites, the active, motile feeding stage between merozoite and mature schizont. The youngest trophozoites appear as small ring forms. Older mature trophozoites are much larger and usually more compact, and nearly fill the erythrocyte. Within an erythrocyte, the growing trophozoite metabolizes hemoglobin. Pigment granules derived from this process form in the cytoplasm of the growing parasite. In the mature trophozoite, the chromatin mass undergoes asexual division (schizogony), followed by division of the cytoplasm. Complete division of chromatin and cytoplasm produces a mature schizont composed of merozoites and 1 or 2 clumps of pigment. Each merozoite is composed of a small chromatin mass within a tiny fragment of cytoplasm. Eventually, the parasitized erythrocyte ruptures, releasing merozoites into the bloodstream. The released merozoites invade other erythrocytes and commence another round of asexual reproduction.

Not all merozoites that invade erythrocytes evolve into schizonts. Some develop into micro-and macrogametocytes that circulate in the blood until ingestion by a female *Anopheles* mosquito once again triggers the sexual reproductive cycle.

Malaria has 3 known routes of transmission: mosquito-borne, congenital, and blood-borne. When the route of transmission cannot be established, the infection is classified as cryptic malaria. A large majority of infections are transmitted by the bite of an infected female *Anopheles* mosquito (Fig 10.33). Infection can also be acquired in utero or during birth. Blood-borne malaria can be transmitted accidentally through blood-product transfusion, contaminated injection equipment, or organ transplantation. It can also be transmitted intentionally as malariotherapy, widely employed until the 1950s as a treatment for late neurosyphilis. Malariotherapy has recently resurfaced as an unproven alternative treatment for diseases such as AIDS, Lyme disease, and breast cancer.

Clinical Features and Pathogenesis

Malaria has protean clinical manifestations and may mimic almost any disease (Table 10.2). Proerythrodromal symptoms are vague and include lassitude, myalgia, irritability, anorexia, headache, various gastrointestinal symptoms, and chills. Some of the clinical features of malaria are the same for infections caused by all 4 species of *Plasmodium*. For example, many patients develop splenomegaly (Fig 10.37).

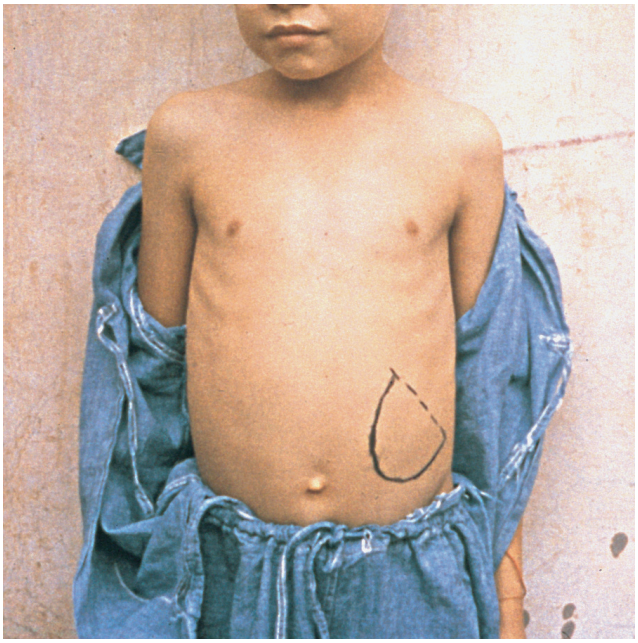

Fig 10.37
Child with malarial splenomegaly. Patient was also anemic.

Table 10.2
Clinical features of malaria caused by *Plasmodium* sp infecting humans.

	P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Complications	Very rare transient cerebral irritation	Severe anemia, frequent cerebral, pulmonary, and renal complications	Rare progressive glomerulonephritis	Milder than *P. vivax*
Splenomegaly	Less severe than *P. falciparum*, rupture 2 to 3 months after infection.	Frequent	Less severe than *P. falciparum*	Same as *P. vivax*
Hepatomegaly	Less severe than *P. falciparum*	Frequent, with jaundice	Very rare	Same as *P. vivax*
Other features	Late-day paroxysms, high fever, nausea, vomiting	High fever, nausea, vomiting, diarrhea	Peripheral edema	Same as *P. vivax*

In long-standing chronic malaria or with repeated attacks, the spleen may become enormously enlarged, a condition sometimes called tropical splenomegaly syndrome or big spleen disease. Traumatic splenic rupture can cause death. Asplenia results in rapid progression of disease and high parasitemia.

The classic symptom common to all malarial infections is cyclic fever. Fever peaks around the time of schizogony and is more severe in naive patients than in those who have had previous infections. Malarial pyrogens may be products of ruptured erythrocytes or toxic products of the parasites themselves. When parasites develop synchronously, the great majority undergo schizogony at approximately the same time and fever periodicity is determined by the length of the asexual cycle (Table 10.3). Malarial paroxysms are usually sudden, with 3 discernible stages: chills, fever, and sweating. After the paroxysm, the exhausted patient usually sleeps.

Temporal features of malaria vary with factors such as host response and the species of *Plasmodium*. The prepatent period between inoculation and the appearance of parasites in peripheral blood can be lengthened by immunity and prophylactic drugs. The incubation period between inoculation and the appearance of clinical symptoms varies depending on the degree of acquired immunity and the number of sporozoites inoculated. In all forms of malaria, clinical symptoms usually appear 2 or 3 days after parasites appear in peripheral blood. In tertian malaria caused by *P. vivax*, *P. falciparum*, or *P. ovale*, the asexual cycle takes 48 hours, resulting in fever every third day. In quartan malaria caused by *P. malariae*, the asexual cycle takes 72 hours, resulting in fever every fourth day. However, fever periodicity is not a reliable indicator of the species of *Plasmodium* because the cycle may be erratic in patients with repeated or mixed infections, or with 2 or more asynchronous broods.

Vivax malaria is very rarely fatal. Erythrocytes infected with *P. vivax* are not sequestered in the microcirculation and thus do not cause the complications associated with

Table 10.3
Temporal features of malaria caused by *Plasmodium* sp infecting humans.

	P. vivax	*P. falciparum*	*P. malariae*	*P. ovale*
Prepatent period (days)	10-13	6-12	15-21	10-14
Incubation period (days)	8-20	12-20	18-40	8-19
Periodicity of fever (hours)	48	48	72	48
Time course	Weeks to months, relapses up to 5 years	1-2 years (untreated)	Several months, relapses up to 30 years	Same as *P. vivax*

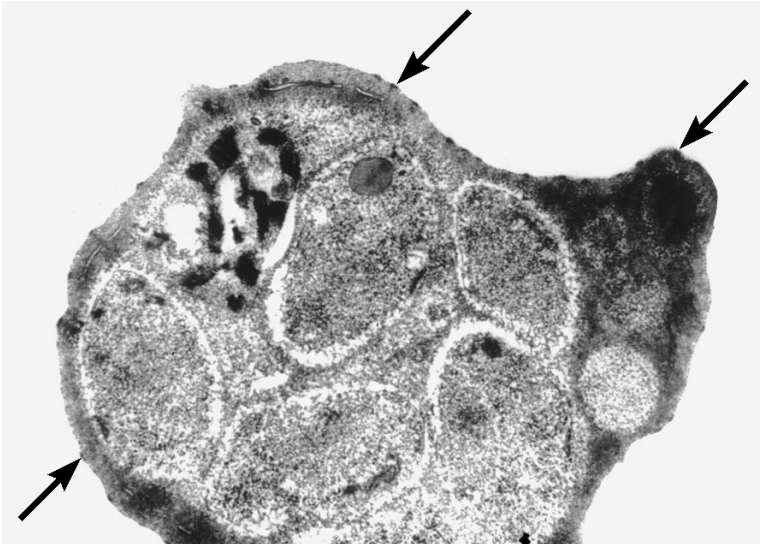

Figure 10.38
Transmission electron micrograph of *Plasmodium falciparum* in patient with ocular malaria. Note knobs, conical evaginations, on surface of erythrocyte (arrows).

falciparum malaria. *Plasmodium vivax* infects only young erythrocytes; however, hemolysis-induced hematopoiesis can cause significant parasitemia. Relapses of *vivax* malaria occur when merozoites are released into circulation from hepatic hypnozoites. Relapses are usually less severe and of shorter duration than the primary attack, but still create a considerable economic burden for individuals and society. Many Africans are resistant to *P. vivax* infection because they lack the Duffy blood group antigens that are the receptors for the merozoites.

Plasmodium falciparum has several unique features that make it the most virulent species and the one that accounts for most deaths due to malaria. It has no secondary exoerythrocytic stage, it parasitizes erythrocytes of any age, and its preerythrocytic schizonts release many more merozoites than other *Plasmodium* sp. Knobs on parasitized erythrocytes facilitate adherence to capillary endothelium (Fig 10.38). Because parasitized erythrocytes are sequestered in the microcirculation, some heavily infected patients have few circulating parasites.

Falciparum malaria often begins with severe gastrointestinal symptoms. Later, patients may develop high fever, hepatomegaly, hepatic tenderness, jaundice due to hemolysis, and splenic tenderness. Laboratory tests may show slightly elevated levels of bilirubin and transaminases. Relapses do not occur because there are no hepatic hypnozoites and because erythrocytic parasites do not persist longer than 1 or 2 years. Fatal complications, usually resulting from microvascular disease, occur more frequently in patients who are very young, immunodeficient, or pregnant, or who have high parasitemia.[7,11] "Algid malaria" describes the clinical findings of clammy skin, cyanosis, and hypotension due to circulatory collapse.[12] Signs and symptoms of cerebral malaria include headache, nuchal rigidity, altered levels of consciousness, seizures, ataxia, aphasia, and dysarthria. Renal involvement may take the form of glomerulonephritis with proteinuria, hemoglobinuria, oliguria, or abnormal urinary sediment. Acute intravascular hemolysis can cause hemoglobinuric nephrosis with renal failure. "Blackwater fever" describes the appearance of dark urine after an acute attack of falciparum malaria. Other complications include gastroenteritis in children, pulmonary edema, severe normocytic anemia, hypoglycemia, and disseminated intravascular coagulopathy. Falciparum malaria during pregnancy increases the risk of maternal anemia, fetal death, prematurity, intrauterine growth retardation, and low birth weight.[7] Falciparum malaria is less severe in heterozygotes for the sickle hemoglobin gene.

In individuals with some acquired immunity to malaria, destruction of pre-erythrocytic stages in the liver by cytotoxic T cells or other mechanisms can prevent the development of disease.[13] If parasites do enter the blood, antibodies may control parasite density and severity of infection.[14] Also, immune responses directed at certain parasite components, such as those involved in fever or adherence to endothelial cells, may help to modify the clinical response.[15] In cerebral malaria there appears to be a complex interaction between pro-inflammatory and anti-inflammatory cytokines affecting sequestration of circulating blood cells[16,17] Parasite-derived molecules—surfaces or soluble—remain necessary but not sufficient to explain the development of cerebral malaria.[18] Persons exposed frequently to malaria have increased immunoglobulin production.[19] Chronic B-cell activation is probably a consequence of immune responses to malarial antigens and of nonspecific B-cell stimulation by toxins and mitogens.[20]

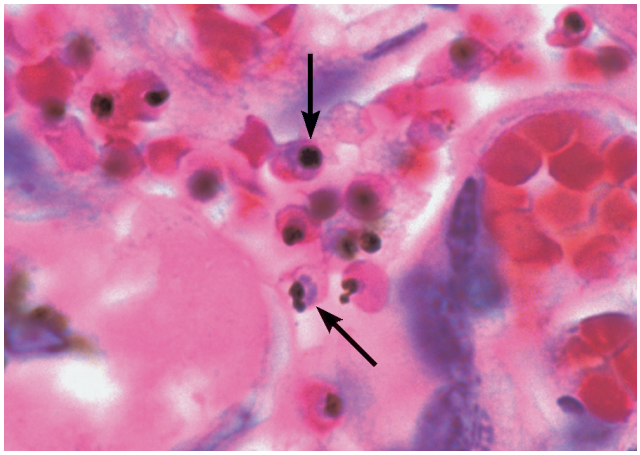

Figure 10.39
Plasmodium falciparum trophozoites in maternal erythrocytes in placenta. Note pigment and blue cytoplasm (arrows). x1255

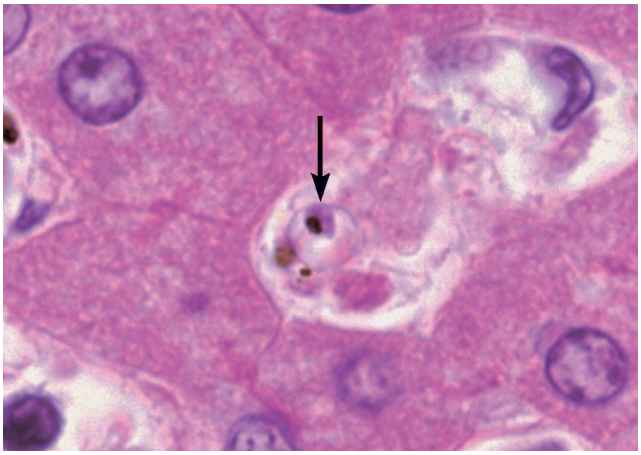

Figure 10.40
Trophozoite in Kupffer's cell in liver. Note pigment and cytoplasm (arrow). x1485

AIDS may affect some components of the immune response to *P. falciparum*.[21] After adults leave an endemic area, their protective immunity wanes slowly. It is therefore likely that established antibody responses will be sustained for several years in patients with low CD4 cell counts. In young children, CD4 cells probably play a vital role in the development of immunity to malaria.[22]

Like vivax malaria, *P. malariae* infection is not complicated by microvascular disease. Quartan malaria often has an insidious onset and may cause leukopenia and mild anemia. Relapses do not occur because there are no hepatic hypnozoites. Recrudescence of persistent erythrocytic parasites causes recurrence. Protracted low-grade asymptomatic parasitemia may persist for up to 30 years, making carriers a source of transfusion malaria. Continued antigen stimulation associated with persistent parasitemia may cause immune complex glomerulonephritis. Relapses also occur in ovale malaria due to hepatic hypnozoites.

Clinical features of ovale malaria are similar to those of vivax malaria. Symptoms tend to be mild, and fever often subsides after a few cycles with or without treatment.

Pathologic Features

Knobs on the surface of erythrocytes parasitized by *P. falciparum* adhere to surface receptors on capillary endothelial cells, believed to be thrombospondin, CD36, and ICAM-1 (Fig 10.38).[15,23] Because *P. falciparum* is the only malarial parasite that becomes sequestered in vessels, it is the only species of *Plasmodium* that we have observed in tissue sections.

In hematoxylin and eosin-stained sections, trophozoites of *P. falciparum* appear as small (2 µm to 4 µm), usually spherical masses of cytoplasm, each containing at least one round clump of dark, birefringent pigment (Figs 10.39 & 10.40). Within an erythrocyte, where trophozoites are most commonly found, the parasite cytoplasm usually stains poorly and may not be visible (Fig 10.41). Infected erythrocytes are frequently depleted of hemoglobin (Fig 10.42), frequently, only malarial pigment can be identified (Fig

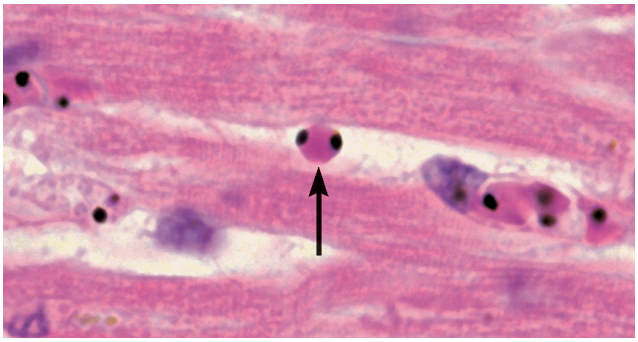

Figure 10.41
Two clumps of malarial pigment in erythrocyte (arrow) in myocardium. Parasite cytoplasm not visible in photo. x955

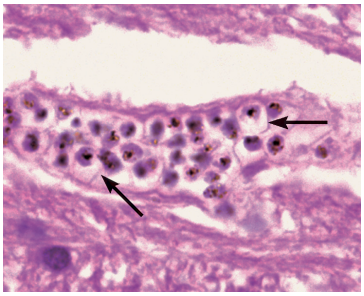

Figure 10.42
Trophozoites with cytoplasm and pigment in hemoglobin-depleted erythrocytes (arrows) in cerebral capillary. x960

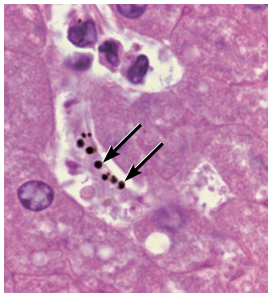

Figure 10.43
Spherical clumps of malarial pigment in Kupffer's cell in liver. x870

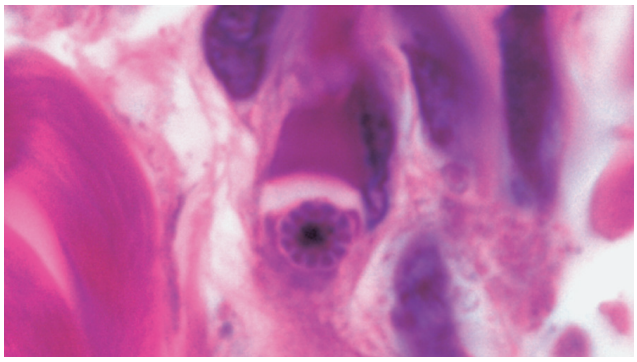

Figure 10.44
Schizont in erythrocyte in dermal blood vessel. Note round clump of pigment and merozoites in rosette pattern. Original magnification x500

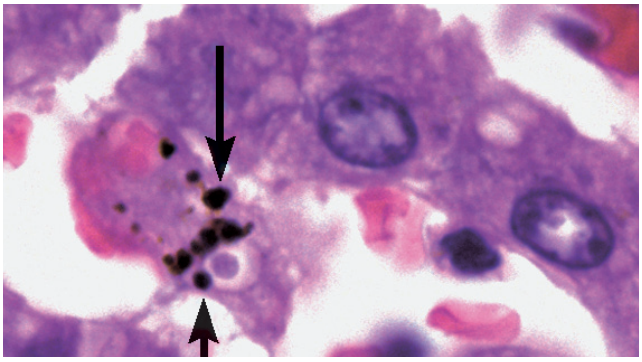

Figure 10.45
Round clumps of malarial pigment (arrows) in Kupffer's cell in liver. Elongate aggregate between arrows is also malarial pigment. Original magnification x250

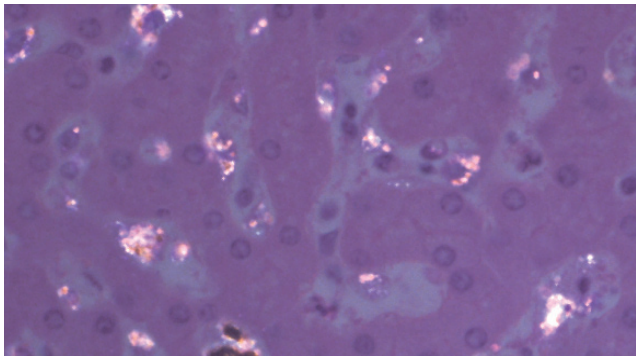

Figure 10.46
Birefringent malarial pigment in liver viewed under polarized light. x250

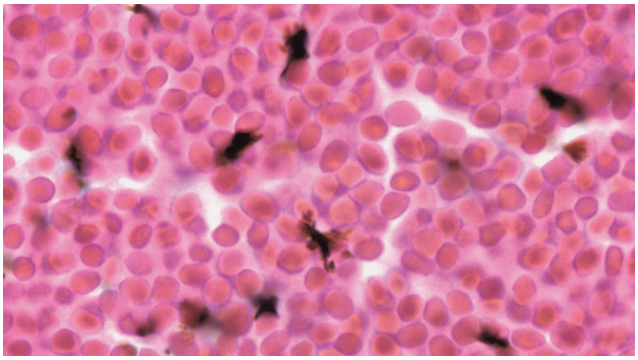

Figure 10.47
Large clumps of extracellular nonmalarial pigment in vessel containing erythrocytes. Note that pigment clumps are not round. x800

10.43). Schizonts of *P. falciparum* are occasionally observed in tissue sections. They are usually spherical, 4 µm to 5 µm in diameter, and have a clump of pigment surrounded by tiny merozoites in a rosette pattern (Fig 10.44). Gametocytes are rarely seen in tissue sections.

Malarial pigment is the end product of hemoglobin digestion into a porphyrin conjugated with a protein derived from the globin portion of hemoglobin. Malarial pigment and formalin pigment are morphologically very similar. Both appear microscopically as brown or black crystals that are birefringent under polarized light. However, they can usually be distinguished by certain general characteristics: malarial pigment appears as intracellular round granules (Figs 10.45 & 10.46); formalin pigment is extracellular and frequently rod-shaped (Figs 10.47 & 10.48).

The following steps help to prevent deposition of formalin pigment in congested tissues:

- Trim tissue to less than 3 mm thick.
- Immediately place tissue in phosphate-buffered neu-

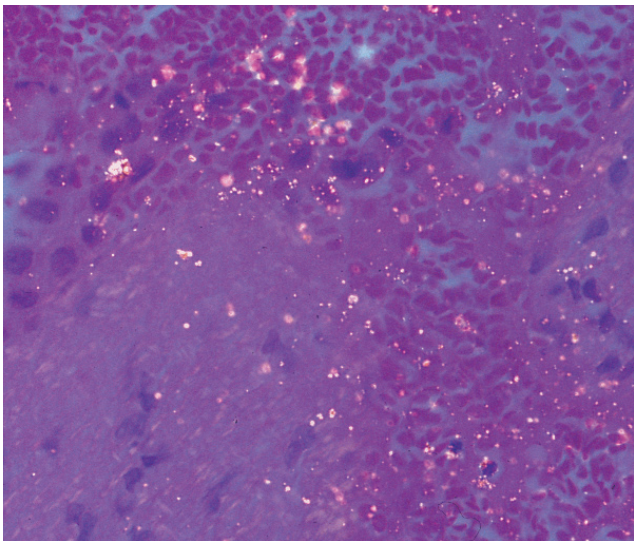

Figure 10.48
Birefringent nonmalarial pigment in lung viewed under polarized light. Original magnification x300

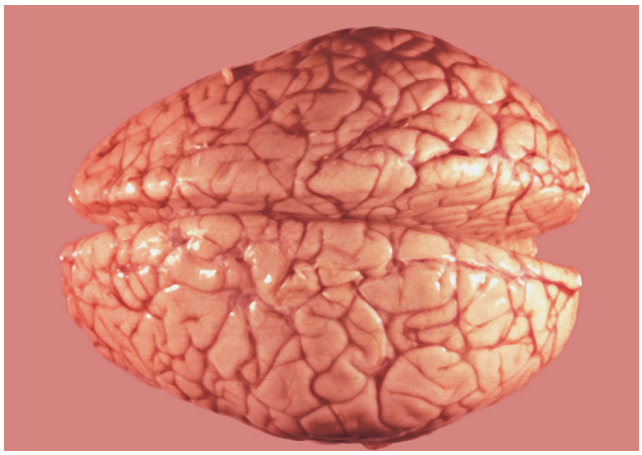

Figure 10.49
Brain of child who died of falciparum malaria, showing flat gyri and pink discoloration caused by engorged meningeal vessels.

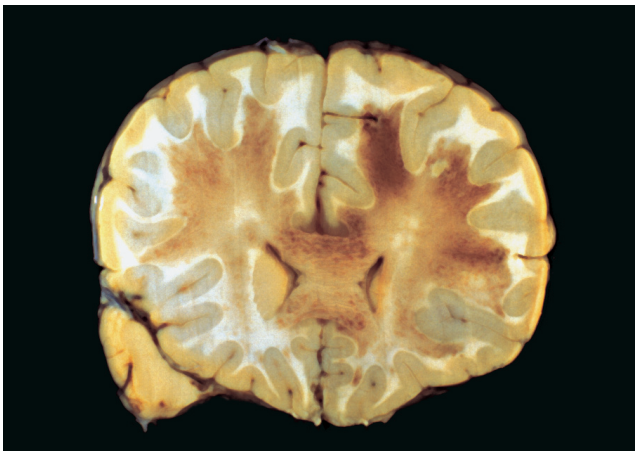

Figure 10.50
Brain of patient who died of falciparum malaria, demonstrating congestion and petechial hemorrhage in white matter.

tral formalin.

- Refrigerate fixative and tissues for the first 24 hours.
- Change the formalin whenever it becomes even slightly discolored.

Postmortem findings of falciparum malaria usually include prominent generalized passive congestion. Grossly, there is often gray or brown discoloration of the brain, liver, and spleen caused by malarial pigment.[24]

The brain is edematous with broad, flat gyri (Fig 10.49). Congested arachnoid vessels give it a pinkish cast. When the swollen brain herniates, there may be grooving of the uncinate and cingulate gyri or cerebellar tonsils. The cut surface shows selective congestion and petechial hemorrhage of white matter (Fig 10.50).

Microscopically, masses of erythrocytes fill the lumina of small and medium-sized blood vessels, distending the vessels. Parasitized erythrocytes tend to lie against the endothelial surface of vessels (Fig 10.51). In a ring hemorrhage, a small vessel is occluded by parasitized erythrocytes, surrounded by necrotic parenchyma, then surrounded by hemorrhage of nonparasitized erythrocytes (Fig 10.52).

Dürck's nodes are areas of demyelination and glial proliferation surrounding an occluded vessel (Figs 10.53 & 10.54). Ring hemorrhages and Dürck's nodes are seen in the brains of patients with cerebral malaria of 9 to 10 days duration. Anoxia may result in nonspecific congestion, edema, microinfarcts, and

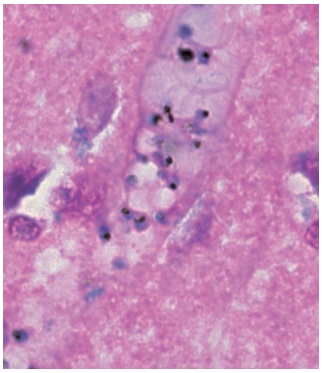

Figure 10.51
Trophozoites with blue-staining cytoplasm and black pigment in cerebral capillary. Parasitized erythrocytes tend to lie against capillary endothelium. Thomas x970

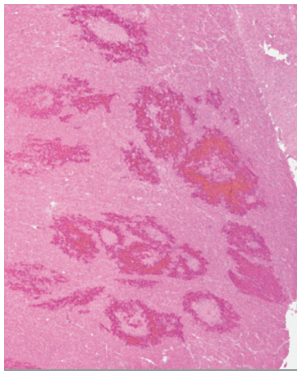

Figure 10.52
Multiple ring hemorrhages in brain. x12

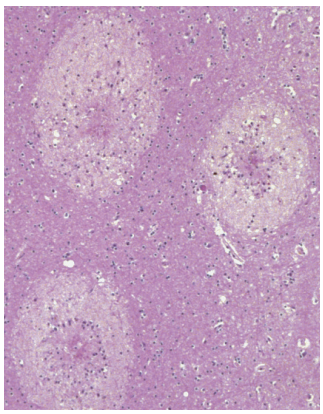

Figure 10.53
Dürck's nodes in cerebellum consisting of central occluded vessel surrounded by demyelination and glial proliferation. x40

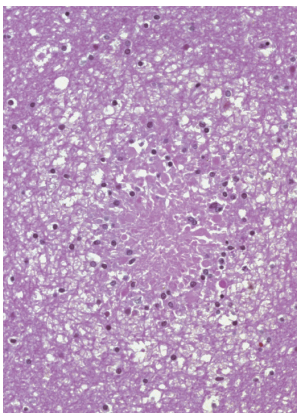

Figure 10.54
Dürck's node in cerebellum showing glial proliferation. x150

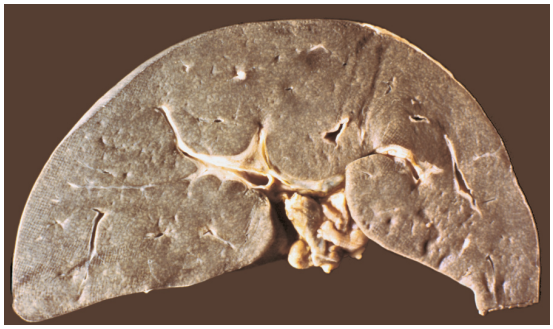

Figure 10.55
Diffuse slate-gray discoloration in liver caused by accumulation of pigment in Kupffer's cells, characteristic of acute falciparum malaria.

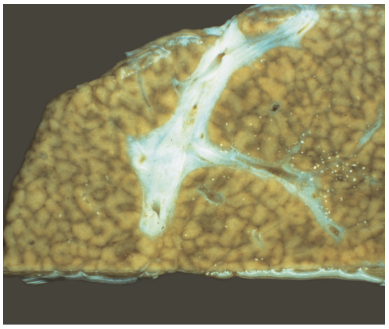

Figure 10.56
Well-delineated lobular pattern in liver caused by pigment deposition in portal areas in chronic falciparum malaria.

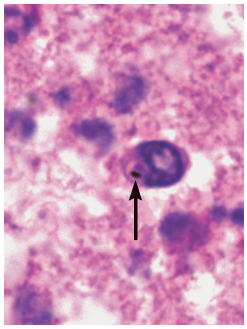

Figure 10.57
Malarial parasite in histiocyte in hepatic sinusoid. Note parasite cytoplasm and round clump of pigment (arrow). x1320

focal hemorrhages.

Grossly, the liver appears gray as a result of accumulated malarial pigment (Fig 10.55). There is an increase in Kupffer's cells, which are enlarged and contain malarial pigment, parasites, erythrocytes, and debris from ruptured erythrocytes (Figs 10.40 & 10.43). Pigment deposition extends to portal areas over time (Fig 10.56). Erythrocytes and histiocytes in the sinusoids may contain parasites (Figs 10.45 & 10.57); parasites and malarial pigment are not seen in hepatocytes. Central veins, sinusoids, and portal vein branches may be congested.

In acute malaria, the spleen is enlarged and darkly pigmented (Fig 10.58). Complications of splenomegaly can include rupture, hemorrhage, torsion, and infarction. The red pulp is congested with parasitized erythrocytes, macrophages containing parasites and malarial pigment, and free parasites (Figs 10.59 to 10.63). Malpighian corpuscles are small and indistinct. In chronic malaria, the spleen is even more enlarged and collections of lymphocytes may appear in the sinusoids. Macrophages containing malarial

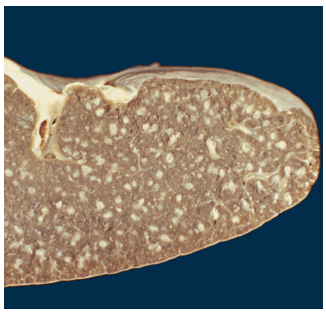

Figure 10.58
Spleen of patient with fatal acute falciparum malaria. Gray discoloration is caused by pigment-laden macrophages.

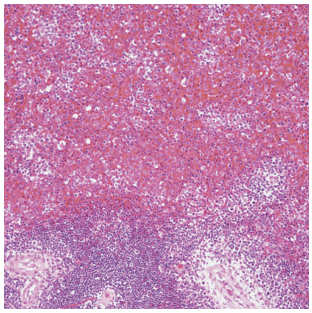

Figure 10.59
Congested spleen of patient with falciparum malaria. x60

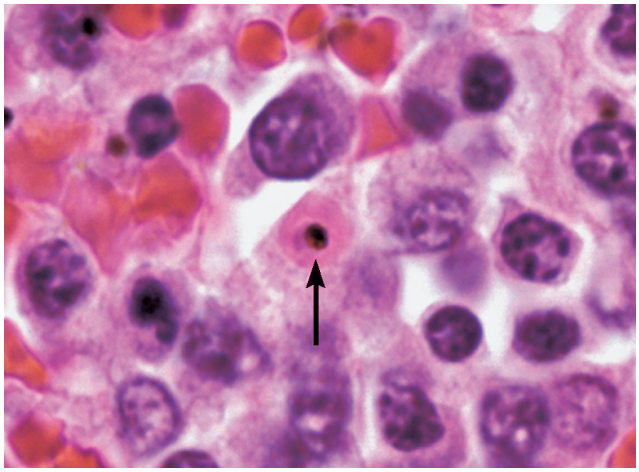

Figure 10.60
Trophozoite in splenic erythrocyte in spleen. Note parasite cytoplasm and round clump of pigment (arrow). x1700

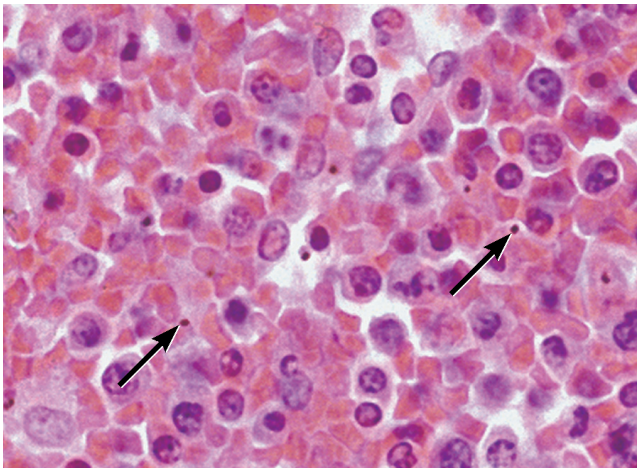

Figure 10.61
Congested spleen with tiny clumps of malarial pigment (arrows). x345

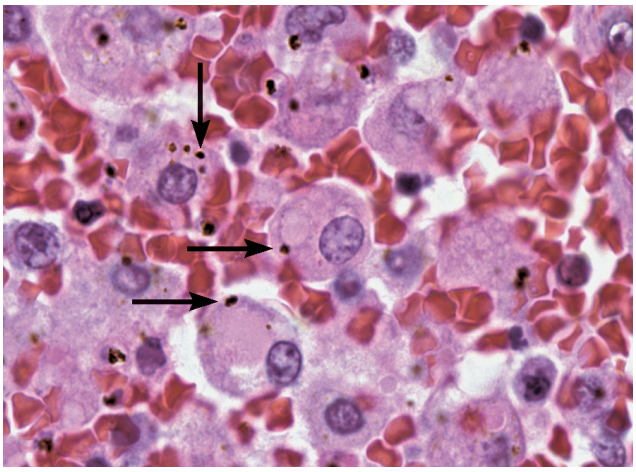

Figure 10.62
Macrophages in spleen containing small round clumps of malarial pigment (arrows). x740

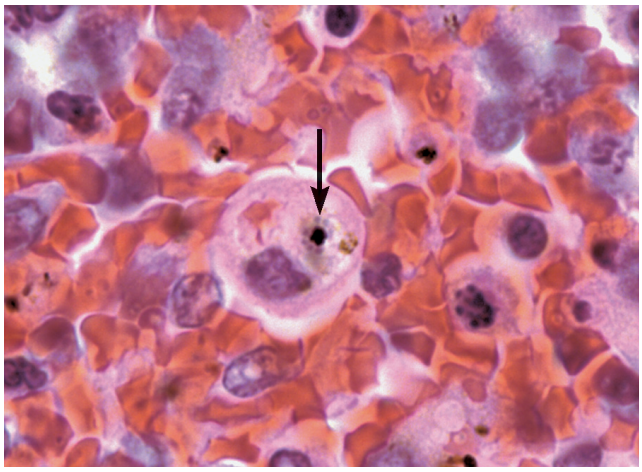

Figure 10.63
Macrophage in spleen containing schizont (arrow). Note round clump of pigment and merozoites in rosette pattern. x1140

pigment are increased, and extracellular pigment grains may coalesce and concentrate along arterioles. White pulp may be depleted. Eventually, fibrosis and foci of mineralization called Gamna-Gandy bodies form with iron deposition (Figs 10.64 & 10.65).

Glomerulonephritis can occur in long-standing or recurrent quartan or falciparum malaria. In mesangiopathic glomerulonephropathy caused by quartan malaria, deposition of immune complexes may be demonstrated by electron or immunofluorescence microscopy. In falciparum malaria, renal corticomedullary capillaries may contain parasitized erythrocytes (Fig 10.66). Pigment grains may be seen within glomeruli or phagocytic cells (Fig 10.67). In patients with oliguria, tubular necrosis and hemoglobin casts may be prominent. In patients with blackwater fever, tubules contain hemoglobin casts and erythrocytic debris, and the

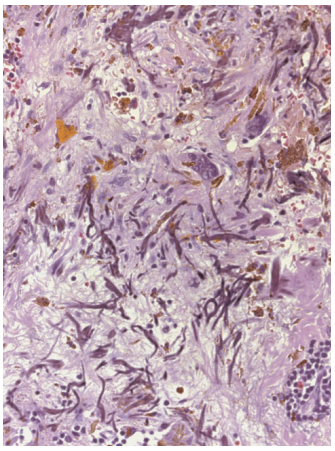

Figure 10.64
Gamna-Gandy body in spleen. x125

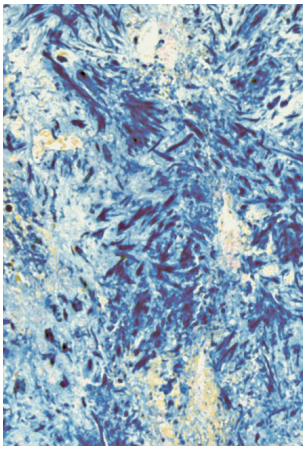

Figure 10.65
Iron deposition in Gamna-Gandy body in spleen. Iron x625

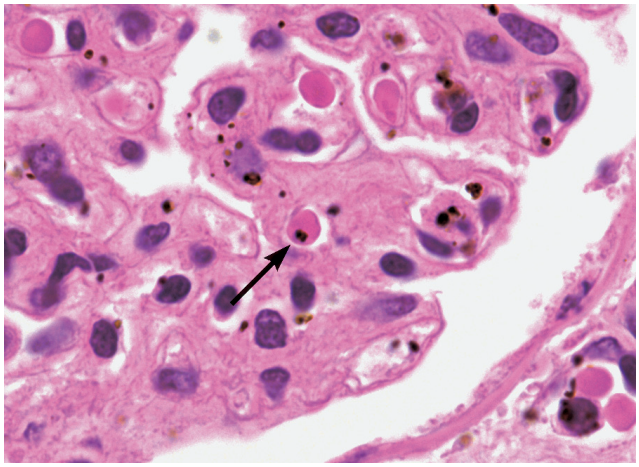

Figure 10.66
Malarial pigment in parasitized erythrocyte (arrow) in renal glomerular capillaries. x775

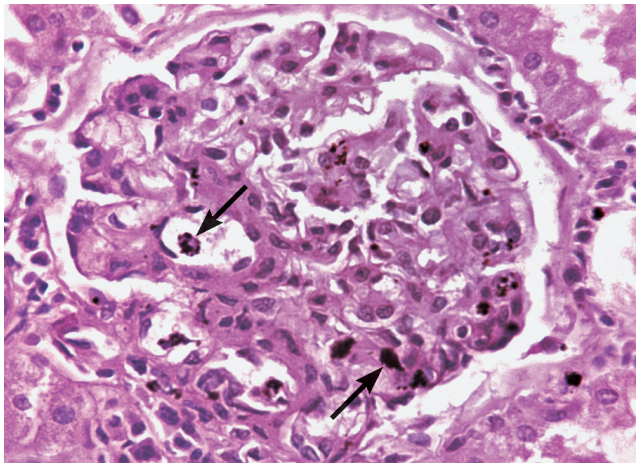

Figure 10.67
Renal glomerulus containing malarial pigment (arrows). x440

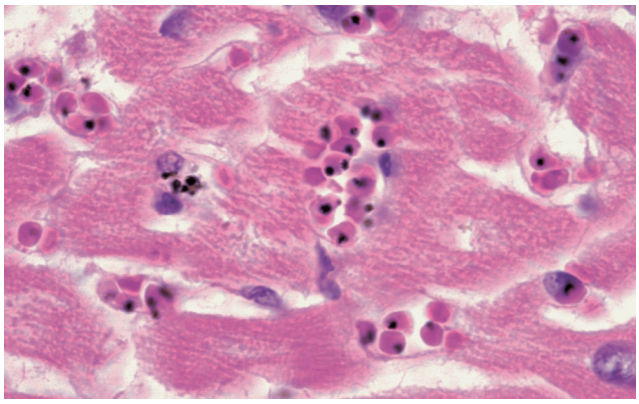

Figure 10.68
Capillary in myocardium occluded by parasitized erythrocytes. x480

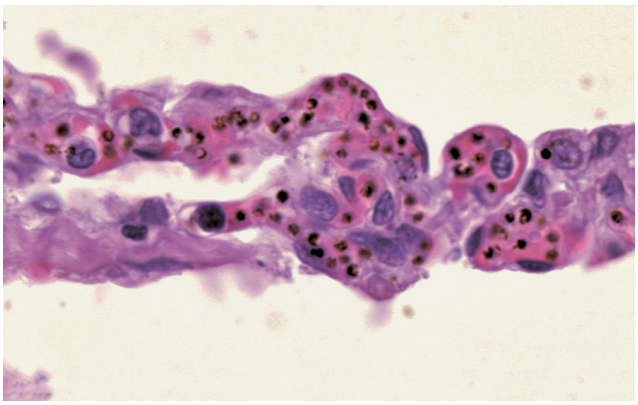

Figure 10.69
Parasitized erythrocytes in capillaries of pulmonary alveolar septa. x575

interstitium may contain focal mononuclear cell infiltrates.

Pericardial and endocardial petechiae may be seen in gross specimens. Microscopically, myocardial capillaries are usually congested and occluded with parasitized erythrocytes (Fig 10.68). There may be interstitial edema and focal infiltration by lymphocytes, histiocytes, monocytes, plasma cells, and rare eosinophils.

In the lung, parasitized erythrocytes may be seen within capillaries (Fig 10.69). Alveolar walls may be thickened with chronic inflammatory cells, especially monocytes, lymphocytes, and plasma cells, and alveolar spaces may be filled with proteinaceous fluid, sometimes mixed with erythrocytes.

Involvement of the placenta is more likely in younger women who have had few pregnancies.[11] Possible complications of malarious placenta include decreased placental size, spontaneous abortion, and maternal death. The placenta is severely affected, with numerous sequestered parasites in the intervillous spaces (Fig 10.70). Most parasites are within erythrocytes (Fig 10.71a to 10.71c), but some are found in monocytes (Fig 10.71b) and some are free in the intervillous spaces. Vessels on the fetal side of the placental barrier are usually parasite-free. Rarely, trauma causes mixing of maternal and fetal blood, allowing passage of parasitized cells into fetal circulation, resulting in congenital malaria

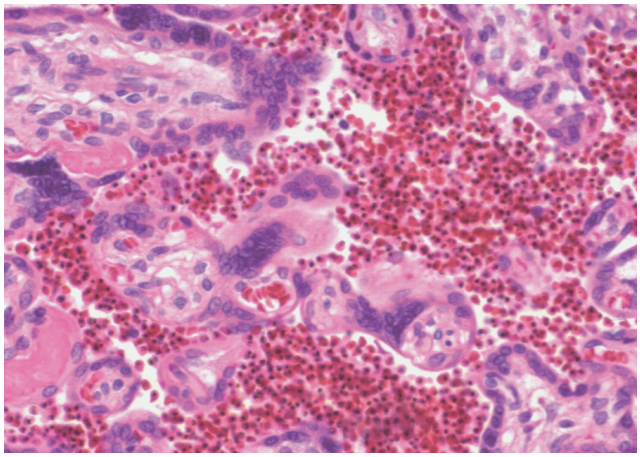

Figure 10.70
Placenta from patient with acute falciparum malaria showing massive parasitization of erythrocytes in intervillous spaces. x245

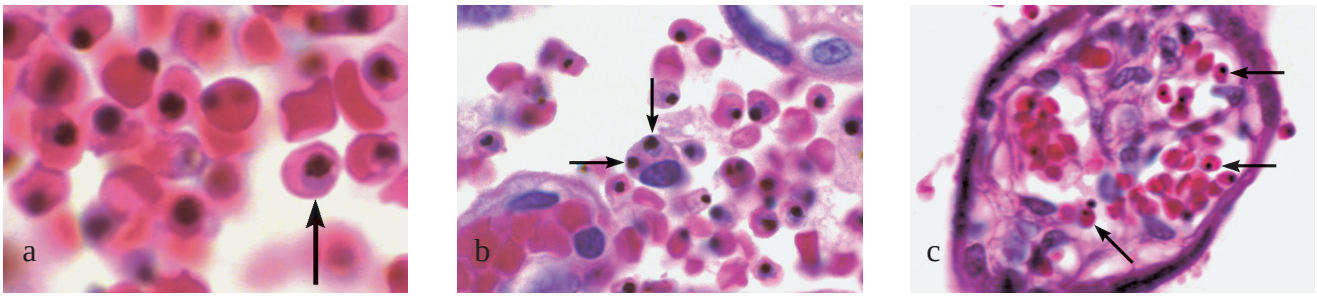

Figure 10.71 a,b,c
Placenta with massive parasitization of maternal erythrocytes. a. Parasite pigment is readily visible; faintly stained parasite cytoplasm (arrow) is more subtle. x1500. b. Two trophozoites (arrows) in mononuclear cell in intervillous space. Note absence of parasites in fetal circulation (lower left). x1250. c. Placenta with several parasitized erythrocytes (arrows) in villus of fetal circulation, a rare finding. x810.

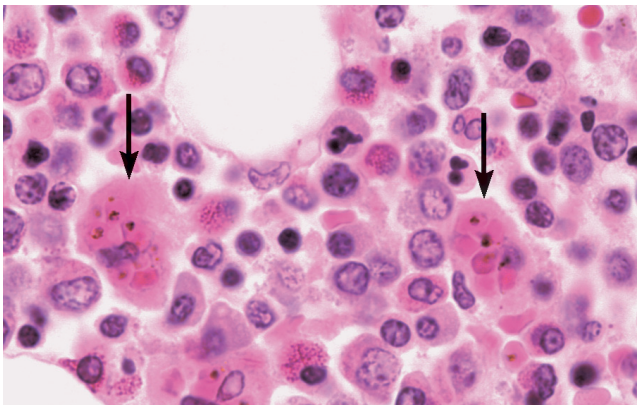

Figure 10.72
Macrophages in bone marrow containing parasitized erythrocytes (arrows). x250

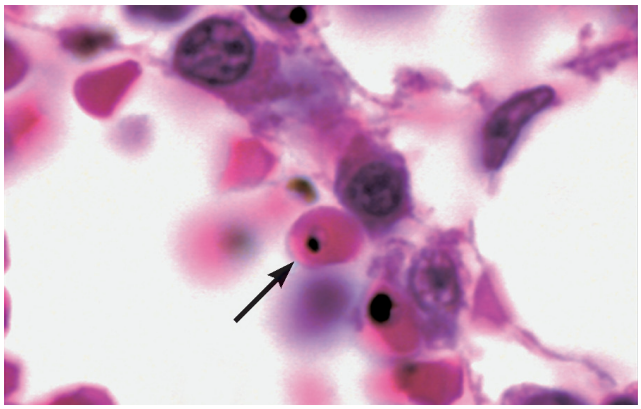

Figure 10.73
Bone marrow with parasitized erythrocytes. Note parasite pigment and cytoplasm (arrow). x300

(Fig 10.71c).

Bone marrow may be hypercellular, with hyperplasia of both erythrocytic and leukocytic precursors. Malarial parasites and their pigments are present in phagocytic cells (Fig 10.72), and congested blood spaces may contain large numbers of parasitized erythrocytes (Fig 10.73). Smears of bone marrow may also demonstrate malarial parasites (Fig 10.74).

As in the spleen, lymph nodes are congested with parasitized erythrocytes, but pigment accumulation in macrophages is less than that in the spleen or liver. Lymphoid follicles tend to be inconspicuous, but there is usually prominent histiocytic hyperplasia.

Edema, congestion, hemorrhage, and parasitized erythrocytes within small vessels may be seen in any tissue, including adrenal gland, gastrointestinal tract (Fig 10.75), skin, adipose tissue, retina[25] (Fig 10.76), parathyroid (Fig 10.77), and skeletal muscle, especially in patients with high parasitemia.

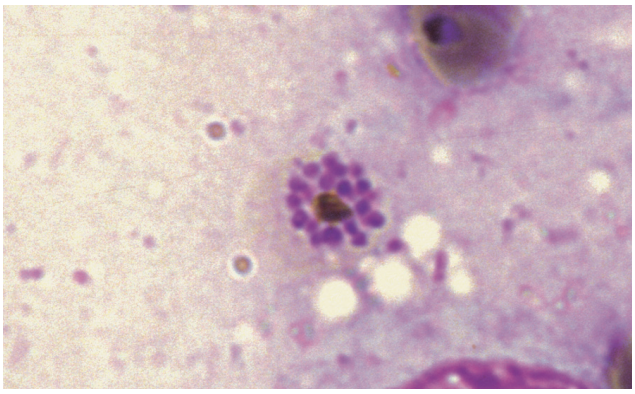

Figure 10.74
Smear of bone marrow showing mature schizont with pigment and merozoites in rosette pattern. Dif-Quick x400

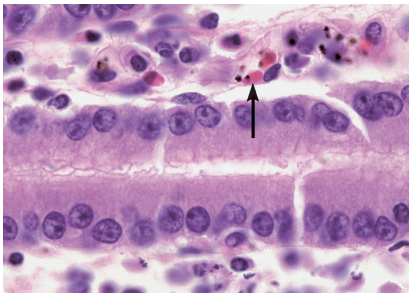

Figure 10.75
Mucosal capillaries of stomach occluded by parasitized erythrocytes. Original magnification x250

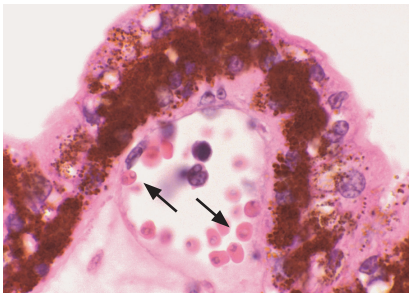

Figure 10.76
Parasitized erythrocytes (arrows) in retinal capillary from 53-year-old patient who died of chloroquine-resistant falciparum malaria. x600

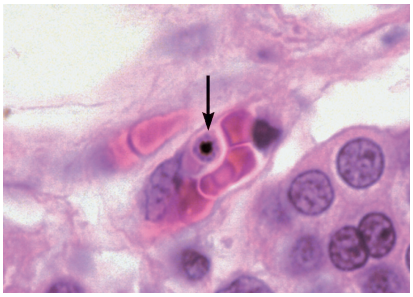

Figure 10.77
Mature schizont (arrow) in capillary in parathyroid gland .Note dark round clump of pigment and merozoites in rosette pattern. x1550

Diagnosis

Diagnosis of malaria is usually established in the laboratory by direct examination of thick or thin peripheral blood films (Table 10.1).[26] Microscopic diagnosis is relatively cost-effective and has a sensitivity of approximately 50 parasites per ml of blood. A highly skilled microscopist can estimate the level of parasitemia and identify all species of *Plasmodium*, based on pertinent factors such as morphologic appearance of the parasite and erythrocytes, geographic considerations, and clinical symptoms. Specific diagnosis depends on scrutinizing blood films and accurately identifying all observable forms of the parasite, determining the degree of development of an observed morphologic feature, and how often it occurs.

Because most morphologic forms of malarial parasites and configurations of erythrocytes can be found in all 4 types of malaria, and a given specimen rarely demonstrates all the classic features of a single species, the most useful diagnostic approach combines comparison and exclusion.

For example:

- Erythrocytes enlarged: If a large number of parasitized erythrocytes are oval or fimbriated, the species is likely to be *P. ovale*. A finding of very few oval or fimbriated parasitized erythrocytes suggests *P. vivax*.
- Band erythrocytes enlarged: All 4 species of *Plasmodium* have thin band trophozoites, but only those of *P. malariae* are large enough to occupy 50% to 75% of the erythrocyte.
- Large numbers of ring trophozoites: If the examiner sees only a large number of ring trophozoites, the species is most likely *P. falciparum*.
- If only a few ring trophozoites are observed, it may not be possible to determine the species, and the specimen should be signed out as "malaria, species undetermined."
- In *vivax*, quartan, and *ovale* malaria, parasitemia is usually less than 2%. In *falciparum* malaria, parasitemia may be 40% or higher.
- If parasitized erythrocytes are enlarged, quartan malaria can be excluded, except in mixed infections.
- If gametocytes are round, *falciparum* malaria can be excluded.

Some authorities believe that parasite morphology may be affected by geographic location, parasite strain, and the patient's age, immune status, and treatment.[27] Investigating the patient's travel history may provide clues to the species of *Plasmodium*. Although morphologic features of the parasite are of greater diagnostic value than travel history, a thorough examiner must determine where the patient has been and what species have been reported in those areas.

Mixed infections are probably underdiagnosed. When one species predominates in a blood film, the other species is easily overlooked. Furthermore, upon diagnosing one species of malaria, the microscopist usually looks no further. To diagnose a second species, one of its diagnostic forms must also be identified. All 4 species of *Plasmodium* have diagnostic forms:

- *vivax*: ameboid trophozoite in enlarged erythrocyte with Schüffner's dots.
- *falciparum*: crescent-shaped gametocyte.
- *malariae*: broad band trophozoite.
- *ovale*: oval, fimbriated, enlarged erythrocytes.
- Mature schizonts of all 4 species are usually diagnostic when other stages are also present.

Preparation and Examination of Peripheral Blood Films

The biggest pitfall in most laboratories in developed countries is leaving too great a delay between taking the blood sample and making the blood films. As blood cools to room temperature, male gametocytes will divide and release microgametes: these are long sinuous filamentous structures that can be mistaken for organisms such as *Borrelia*. If the blood is kept at warmer temperatures, schizonts will rupture and merozoites invading erythrocytes will mistakenly give the appearance of the appliqué form of *P. falciparum*. If *P. vivax* or *P. ovale* is left for several hours in EDTA, the build up of acid in the sample will cause the parasitized erythrocytes to shrink and the parasite will roll up, simulating the appearance of *P. malariae*. This problem is made worse if anticoagulants such as heparin or citrate are used. The anticoagulant that causes the least problems is EDTA. Romanowsky stain or a variant stain is usually used. Some laboratories mistakenly use the same staining pH as they do for routine haematology blood films (pH 6.8): malaria blood films must be stained at pH 7.2, or Schüffner's dots and James's dots will not be seen.

Both thick and thin films should be prepared, preferably on the same slide, from any patient suspected of having malaria (Fig 10.78). Thick films are useful for screening because a larger volume of blood is examined and erythrocytes are hemolyzed, concentrating the parasites and possibly revealing multiple stages. For diagnosis, thick films are significantly more sensitive than thin films because 16 to 30 times as much blood is examined. Thick films can be many

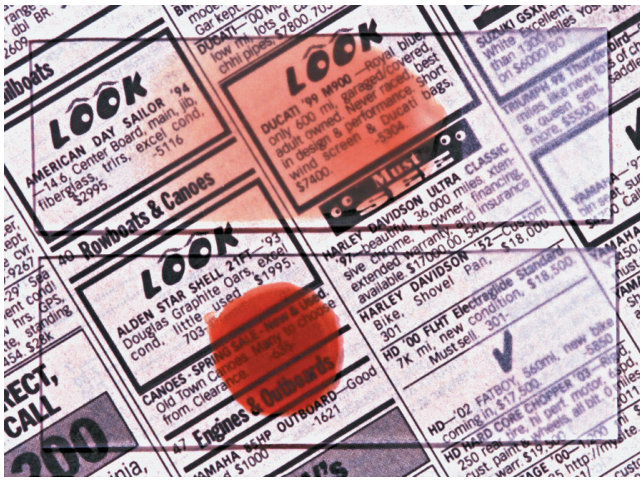

Figure 10.78
Thin (top) and thick blood films prepared on separate slides. Newsprint should be readable through feathered edge of properly prepared thin film. (Typically, in most busy clinics, both thin and thick smears are placed on the same slide.)

cell layers thick and are never fixed, since fixation prevents dehemoglobinization. The examiner should use the 100x oil immersion objective to study thick films, and should search for at least 5 minutes (an estimated 100 fields) before reporting a finding of no parasites.

Thin blood films are helpful in species identification because they reveal morphologic details of parasites and erythrocytes not apparent on thick films. On an ideal thin film, the cells are in a single layer and do not overlap. Erythrocytes infected by older parasites are more frequently seen along the feathered edge of the thin film; ring forms are usually evenly distributed. To determine whether parasitized erythrocytes are enlarged or fimbriated, compare them with adjacent uninfected erythrocytes.

Blood films must be carefully prepared and meticulously stained.[28] Slides should be spotlessly clean and free of chemicals, grease, dust, and scratches. Thin films should be fixed in pure methyl alcohol before staining. Ideally, films should be stained within 24 hours, and no later than 72 hours, before the blood loses its affinity for stain. Although Wright's stain can be used for thin films, the most dependable stain for visualizing malarial parasites is Giemsa diluted with distilled water and buffered to pH 7.0 to 7.2. Field's stain is fast and convenient, but the staining process occasionally washes off the entire film.[29]

Erythrocytes on Giemsa-stained thin films should be pale pink and parasites densely stained. Staining artifacts (Fig 10.79) and other structures (Figs 10.80 & 10.81), including platelets (Fig 10.82), may be mistaken for malarial parasites on thin films. To prevent errors, a skilled examiner must

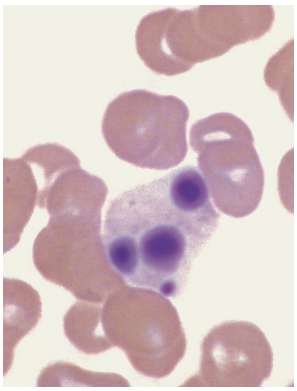

Figure 10.79
Staining artifact, not to be mistaken for malarial parasite. Giemsa x1400

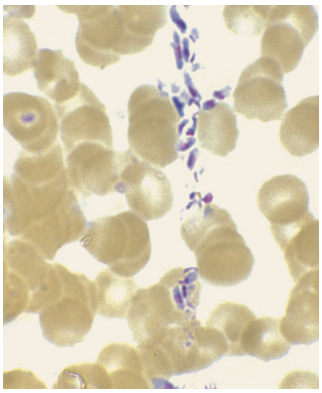

Figure 10.80
Unknown structures in thin peripheral blood film, representing no known human pathogen. Giemsa x825

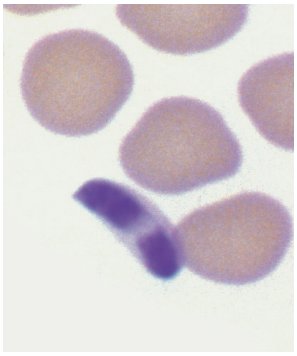

Figure 10.81
Unknown pseudoparasite in thin peripheral blood film, representing no known human pathogen. Giemsa x1775

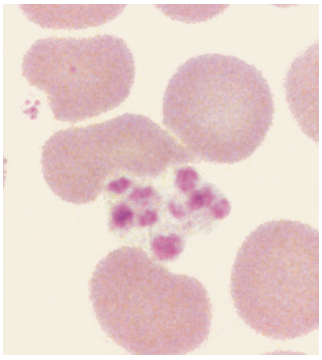

Figure 10.82
Blood platelets in thin peripheral blood film. Giemsa x1600

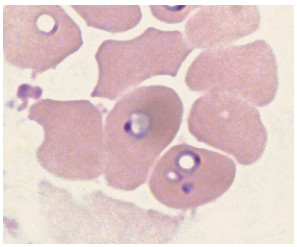

Figure 10.83
Ring trophozoites of *Babesia microti* in thin peripheral blood film. Giemsa x780

be familiar with the appearance of normal blood constituents. Because all stages of the erythrocytic cycle may not be visible in a single smear, repeated examinations should be made at different times of day during the course of infection. If the first blood film is negative, additional thick and thin films should be obtained every 6 hours for 24 hours. Examining blood films under polarized light may simplify

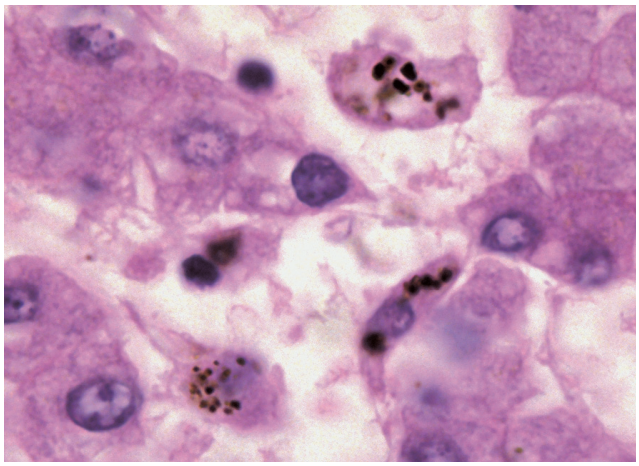

Figure 10.84
Section of liver from 21-year-old Vietnam veteran with acute falciparum malaria. Treatment killed malarial parasites; only remnants of infection are round clumps of malarial pigment in Kupffer's cells. Patient died of staphylococcal infection 6 days post-treatment. x965

and accelerate screening for malarial parasites.

Plasmodium sp must be differentiated from *Babesia* sp, protozoa that also invade erythrocytes and closely resemble malarial parasites (Fig 10.83). *Babesia* sp are round, rod-shaped, piriform, or ameboid, but lack pigment. Parasitized erythrocytes are normal in size. Patients with babesiosis have no schizonts or gametocytes in peripheral blood (see Topic 11 on Babesiosis).

Other Methods of Diagnosis

Postmortem, a diagnosis of malaria is made by identifying malarial parasites or their pigment in histologic sections. (Morphologic features are described above and in Figures 10.40 to 10.77.) Adequate treatment for malaria usually eliminates parasites from the patient, but malarial pigment accumulates in the liver and is readily observable at autopsy (Fig 10.84). Absence of malarial pigment in the liver at autopsy suggests that there was no recent attack of acute falciparum malaria.

Electron microscopy is not commonly used for diagnosis, but can reveal characteristic knobs on the surface of an erythrocyte parasitized by *P. falciparum* (Fig 10.38).

The quantitative buffy coat (QBC®) technique identifies malarial parasites by fluorescent dye.[30] A glass capillary tube containing fluorescent stain is filled with blood and a

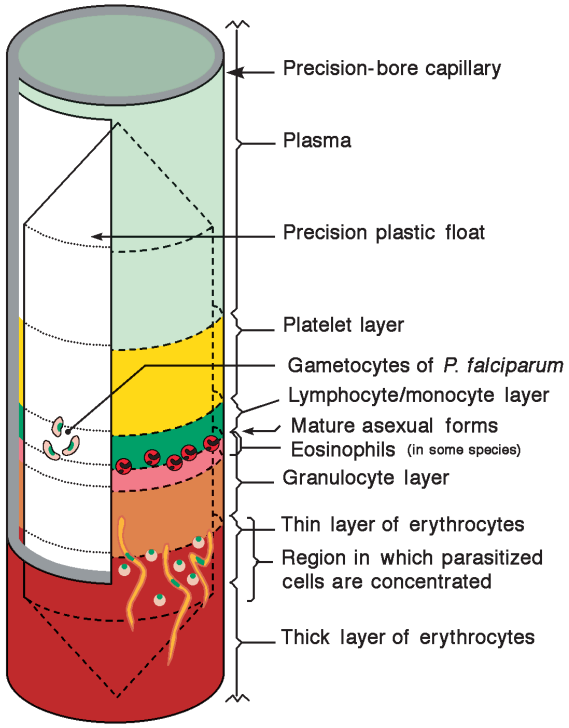

Figure 10.85
QBC® tube for detecting malarial parasites.

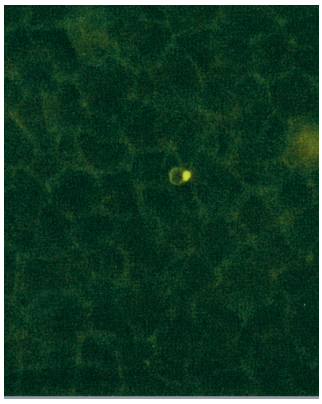

Figure 10.86
Trophozoite of *Plasmodium falciparum* detected by QBC® method. Note well-defined, regularly shaped cytoplasm.

plastic float is inserted (Fig 10.85). When the tube is centrifuged, parasitized erythrocytes localize in the upper layer because of their lower density. Parasites are then detectable by ultraviolet or fluorescence microscopy (Fig 10.86).

Enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA) tests can detect very low densities of malarial antigens in peripheral blood.[31,32] These tests are nearly as sensitive as microscopic techniques and do not require a microscope or an experienced microscopist. Rapid dipstick tests that employ immunochromatographic techniques to detect parasite antigens can provide a crude estimate of the level of parasitemia. These tests have a low turnaround time and do not require skilled personnel or special equipment, but some can detect only one species of *Plasmodium*. Tests targeting histidine-rich protein-2 (ParaSight®-F and ICT Malaria Pf)[29,33,34] detect only *P. falciparum*. OptiMAL® targets lactate dehydrogenase and can distinguish *P. falciparum* from *P. vivax*.[35]

DNA hybridization and PCR methods detect *Plasmodium*-specific DNA sequences in erythrocytes. These assays are often highly sensitive and specific, but may require expensive equipment.[36]

Indirect fluorescent antibody (IFA) test is the preferred method for detecting antimalarial antibodies in serum.[37] Serologic assays cannot distinguish current and prior infections and are used primarily in epidemiological studies.

Treatment and Prevention

Chloroquine (Aralen®) was once the treatment of choice for malaria but is no longer reliably effective outside the Middle East, the Caribbean, and Central America because of resistant *P. falciparum* strains.[38] *Plasmodium vivax* also is chloroquine-resistant in some endemic areas, particularly Papua New Guinea and Indonesia.[39] Chloroquine-resistant malaria can be treated with a variety of other agents, including mefloquine (Lariam®),[38] atovaquone-proguanil (Malarone®),[40] doxycycline, sulfadoxine-pyrimethamine (Fansidar®), halofantrine, and quinine combined with tetracycline. When atovaquone-proguanil is used to treat vivax malaria, it should be followed by primaquine to eradicate persistent liver hypnozoites. Resistance to mefloquine and sulfadoxine-pyrimethamine has become a significant problem in some parts of Southeast Asia and South America.[39] Combining mefloquine with artemisinin derivatives may decrease resistance.[41] Sulfadoxine-pyrimethamine-resistant malaria has been reported in the Amazon Basin, Southeast Asia, and some countries in eastern and southern Africa.

For complicated falciparum malaria, supportive treatment may include management of hypoglycemia, seizures, pulmonary edema, and renal failure. Exchange transfusion is sometimes recommended for nonimmune patients with high parasitemia.

Oral weekly mefloquine may be the best chemoprophylaxis for travelers in areas endemic for chloroquine-resistant *P. falciparum*.[42] Doxycycline is an effective alternative for travelers who cannot tolerate the side effects of mefloquine. Proguanil taken daily in conjunction with weekly chloroquine is an option for pregnant women traveling in sub-Saharan Africa.[38] Weekly oral doses of chloroquine phosphate are effective prophylaxis in areas with chloroquine-sensitive *P. falciparum*. Oral weekly hydroxychloroquine sulfate (Plaquenil®) is an alternative to chloroquine phosphate, and atovaquone-proguanil is useful as prophylaxis. Long-term travelers who have likely been exposed to *P. vivax* or *P. ovale* may be asymptomatic carriers and at risk for later development of malaria. Primaquine phosphate administered orally once daily for 14 days after departure from an endemic area can eliminate a carrier state.[38]

Anopheles mosquitoes bite between dusk and dawn. During waking hours, bites are best prevented by covering most skin with clothing and treating exposed skin with an insect repellent that contains a 30% to 35% concentration of N,N diethyl-meta-toluamide (deet).[38] In sleeping areas that are not screened or air-conditioned, bites can be prevented by sleeping under mosquito netting treated with permethrin or deltamethrin, or by spraying the room with pyrethroid-containing formulas.[43] Other preventive measures include application of environmental mosquito larvicides and drainage of mosquito breeding sites.

Several types of antimalarial vaccine are currently being studied, including cocktails of antigens of asexual blood-stage organisms, DNA recombinant protein, and transmission-blocking vaccines. The search for an effective vaccine is hampered by the parasites' remarkable capacity to vary critical antigenic structures.[44]

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Figure 10.32
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Figure 10.33
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Figure 10.37
Contributed by William Gordon

Figure 10.85
Contributed by SC Wardlaw

Figure 10.86
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